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EXPERIMENTS ON THE ERADICATION OF CANADA THISTLE, CIRSIUM ARVENSE, WITH CHLORATES AND OTHER HERBICIDES[1]

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INTRODUCTION

Canada thistle, *Cirsium arvense* (L.) Scop., is a perennial weed. Its root system penetrates deep into the ground, survives the winter, and forms shoots the following spring. Canada thistle propagates very rapidly and is difficult to eradicate. The experiments herein reported were undertaken in order to develop methods for its eradication with herbicides on arable land.

HISTORICAL REVIEW

The use of herbicides on arable land is a relatively new achievement. The destruction of annual weeds in grain fields was first attempted about 1895, as has been stated elsewhere (*6*).[3] This method is now rather extensively used in some countries. When it was desired to destroy perennial weeds on arable land, the herbicides were either applied to single plants or repeated sprayings were necessary. Thompson and Robbins (*31*) reported good results from the application of several herbicides to barberry bushes, *Berberis vulgaris* L. Gray (*16*), Stewart and Pittman (*30*), and other workers have reported experiments in which herbicides, generally sodium arsenite, was sprayed on perennial weeds. Two or more applications were found necessary for eradication. A practical method for the destruction of perennial weeds on arable land by a single application of a herbicide has not yet been described.

In the following experiments the use of chlorates gave the most satisfactory results. The earliest record of the use of chlorates for the eradication of weeds that has come to the writer's attention dates back to 1901. Potassium chlorate was applied to prickly pear in Australia (*1*). The results seem to have been unsatisfactory. A considerable time elapsed before they were mentioned again, but during the last five or six years they have been tested rather extensively. The new experiments seem to have begun in France. Loyer (*21*) reports some experiments in 1923. However, he mentions the fact that Rabaté recommends the application of 250 kgm. of sodium chlorate per hectare in a 2 per cent solution for the destruction of all vegetation on garden paths, etc.; so that some experiments must have been performed earlier. Loyer (*21*) recommends an application of

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[3] Reference is made by number (italic) to "Literature cited," p. 932.

800 liters of 1 per cent solution of ammonium chlorate (NH_4ClO_3) in grain crops for the destruction of annual weeds. He adds that an application of 25 kgm. per hectare will not injure the grain. Early in 1925 recommendations based on experiments "during the last three years" were published in Denmark (*29*). A 5 per cent solution of sodium chlorate applied at the rate of 6,000 to 8,000 liters per hectare is recommended for the eradication of weeds on garden paths, etc. A 1.5 to 2 per cent solution, at the rate of 1,000 liters per hectare, was recommended for the destruction of annual weeds in grain crops. Korsmo (*18, 19*) reports experiments during 1923–1926. In his latest report (*19*) he states that an application of a 5 per cent solution of sodium chlorate at the rate of 1 liter per square meter killed 11 species of perennial weeds, including Canada thistle. Eight perennial weeds, including quack grass, *Agropyron repens* (L.) Beauv., were not killed by a single application. The experiments were performed on weeds growing along ditches, roadsides, etc. Feilitzen (*11*) recommends a 5 per cent solution of sodium chlorate applied at the rate of 1 liter per square meter for the eradication of all vegetation on tennis courts, etc. In 1924 and 1925 the writer (*3, 4*) used dilute solutions of sodium and potassium chlorate for the eradication of annual weeds in grain crops. The sprays were found to injure the grain almost as much as the weeds. Latshaw and Zahnley (*20*) obtained promising results by using a 12.5 per cent solution of sodium chlorate spray on field bindweed, *Convolvulus arvensis* L. The optimum time for the first application of spray seemed to be about the time the flowers were in full bloom.

THE WORKING HYPOTHESIS

Eradication of perennial weeds must aim at the destruction of the perennial structures. The destruction of the annual growth weakens the plants. Repeated destruction may lead to eradication, as the perennial part becomes exhausted by the forced shoot production. The poisoning of the perennial parts with a herbicide will kill the whole plant.

The perennial part of Canada thistle is its root or root system, the principal part of which consists of the propagation roots which grow more or less horizontally 15 to 30 cm. below the surface of the ground. These roots form numerous shoots.

The poisoning of the root system of Canada thistle may be assumed to be possible in two ways. Herbicides may be applied to the tops or to the roots. It has been found, however, that herbicidal sprays applied to the tops of plants do not easily penetrate into the roots. Schulz and Thompson (*28*) dipped tops or leaves of tomato plants in an arsenical spray solution. It was found that the treated parts died, but the arsenic moved only slightly or not at all to adjacent tissues of the plants. Barberry bushes were not killed when the spray was applied to the foliage. This fact indicates that the poison was not transported to the roots. Gray (*15*) found that spraying mature vines of morning glory within the fog belt with an arsenical spray killed the roots to a depth of 3 to 4 feet below the surface of the ground. Gray (*16*) as well as other workers have failed to obtain such good results under less humid conditions. Some work by Crofts[4] indicates that the reaction of the spray may influence the extent

[4] Oral communication of unpublished data by A. S. Crofts, University of California. 1927.

which it will penetrate the plants. He has found arsenic to a maximum depth of 60 cm. in roots of morning glory when the vines were sprayed with an acid arsenical spray. A considerable number of references dealing with the application of herbicidal sprays on perennial weeds show that a single spraying of the top growth generally causes but little damage. Repeated sprayings have been found necessary. For instance, Munn (*24*) found it necessary to spray dandelions, *Taraxacum officinale* Weber, three to five times during the summer with an 18 to 25 per cent iron sulphate solution in order to obtain eradication or even control. Detmers (*10*) found that single applications of either calcium arsenite or sodium arsenite sprays were insufficient to exterminate Canada thistles from a pasture. These chemicals did not seem to penetrate to the underground parts of the weeds.

Schulz and Thompson (*28*) found that several plants were killed rapidly by the application of sodium arsenite solutions to their roots. Arsenic was found throughout tne plant tissues. Also other experiments show that solutions are more easily carried from the roots toward the top than in the reverse direction. Thus, the application of a poison to the roots seems to be a more reliable method for the eradication of perennial weeds than spraying the tops.

The application of a herbicide to the root system of Canada thistle on arable land involves a consideration of several factors.

1. The herbicide must penetrate the soil freely, for the propagation roots of Canada thistle grow at a depth of 15 to 30 cm.
2. The herbicide must decompose or leach out of the soil in order not to interfere with the normal crop production.
3. The herbicide must be effective in relatively small quantities.

The penetration of chlorates, the herbicides to be tested, seemed to be rather good. Feilitzen (*12*) found that coltsfoot, *Tussilago farfara* L., was killed by an application of a 5 per cent solution of sodium chlorate at the rate of 1.25 liters per square meter. This weed has rootstocks which penetrate the ground to a great depth. Since chlorates are rather unstable compounds, it can be assumed that they disappear rapidly in the soil. Loyer (*21*) found that an application of 45 kgm. of ammonium chlorate per hectare did not harm the succeeding crop. Several workers have reported that chlorates are very effective for the destruction of vegetation on tennis courts, etc. In view of these findings, chlorates seemed to offer promising results as herbicides. However, there appears to be a great discrepancy in the reports of the sensitiveness of the plants to chlorates. In France (*21*) an application of a 2.5 per cent solution of ammonium chlorate for the eradication of annual weeds in grain crops was claimed to be noninjurious to the grain plants. In Denmark (*29*) a 2 per cent solution was recommended for that purpose. The writer (*3, 4*) found a 1 per cent solution to be very injurious to barley in northern Sweden. In all the above trials the amount of the spray was 1,000 liters per hectare and the application was made to spring-sown grain crops. This seemed to indicate that there was a gradient in the action of the chlorates. It appeared that temperature greatly influenced their action, as they seemed to be more active the farther north they were tried.

The amount of chlorates used for the destruction of all vegetation on tennis courts, etc., is generally large. Korsmo (*19*) used 300 to

500 kgm. of sodium chlorate per hectare. Feilitzen (*12*) applied 500 to 1,250 kgm. These applications are heavy enough to prevent the use of chlorates on arable land. The cost would be prohibitive. However, if the effect of the chlorates is greater with low temperature, smaller applications late in the autumn or early in the spring might be found sufficient to eradicate Canada thistle.

There were still other indications of the possibility that small quantities of chlorates would have the desired result, if the application were made at the right time: (1) The penetration through the soil might be facilitated; (2) the root system of Canada thistle might be less resistant during certain seasons than others.

The penetration of the herbicide should take place more rapidly when more water is sinking into the soil. The application of the herbicide on wet soil receiving additional water in the form of precipitation would promote the penetration of the herbicide to the roots of Canada thistle. During late autumn and early spring such climatic conditions may prevail within humid regions.

The work of Lund and Rostrup (*22*) and Paczosky (*25*) shows that a part of the root system of Canada thistle dies during the winter. Thus the application of a poison at that time might be more effective than when plants are in full vigor during the vegetative period. The repeated freezing and thawing of the soil during the winter is another strain on the roots, and a small quantity of a poison might prove fatal to the roots during the prevalence of conditions so unfavorable for the plant.

It is obvious that a herbicide, to be used on arable land, must harm the crop plants to the least possible degree, if it shall be of any praccal value. All fields to be spring sown are bare in autumn. An application of herbicides at that time will give the poison a long time to act before any crop is sown. During the winter the herbicide might leach out of the soil or decompose, so that no injury would be caused to the spring-sown crop.

These considerations prompted the writer to undertake the experiments herein reported. Chlorates (and other herbicides for comparison) were applied in the autumn and at various seasons during the year for the eradication of Canada thistle.

EXPERIMENTAL DATA

PRELIMINARY EXPERIMENTS

A preliminary field experiment started in the autumn of 1925 has been reported previously (*5*). Solutions of sodium chlorate, $NaClO_3$ potassium chlorate, $KClO_3$, and sodium arsenite, $NaHAsO_3$, were sprayed on the ground after frost had killed the tops of the vegetation. The applications were made on permanent grasslands infested with Canada thistle. By the following spring the chlorates had severely injured or eradicated the thistles while the arsenite had been without effect. Figure 1 illustrates the effect of sodium chlorate on Canada thistle.

In order to select additional herbicides to be tested with the chlorates some experiments were conducted during the winter of 1925–26. Cuttings of roots of Canada thistle were planted in pots. When shoots sprouted oats were sown in the pots and when the oat seedlings were about 10 cm. high solutions of herbicides were applied

at the rate of 100 to 500 p. p. m. (parts per million) of dry soil. The following chemicals were used: Sodium chlorate, $NaClO_3$; potassium chlorate, $KClO_3$; sodium perchlorate, $NaClO_4$, potassium sulphide, KS_2; calcium sulphide, CaS_2; sodium cyanide, NaCN; and sodium thiocyanate, NaCNS. It was found that cyanide killed the thistles and oats in 3 days, the thiocyanate killed the plants in 12 to 18 days, depending on the amount applied, while the chlorates killed the thistles in 14 to 20 days. Amounts of 300 p. p. m. or more killed the

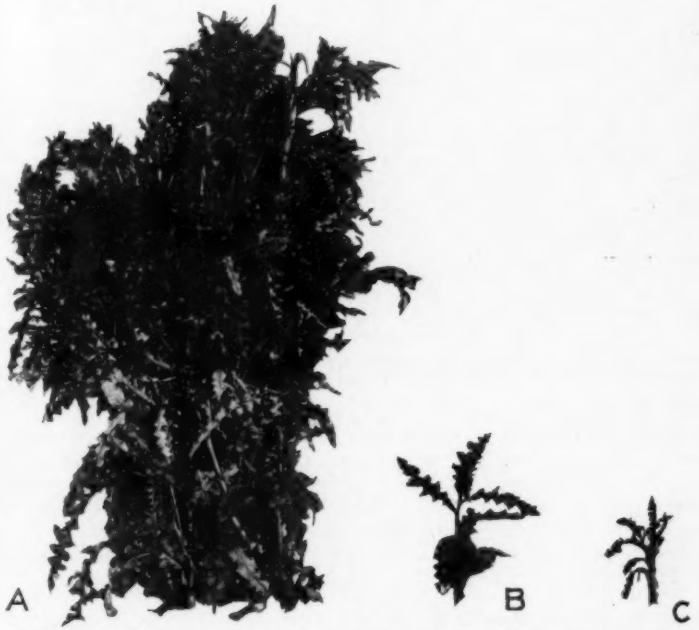

FIG. 1.—Photographs showing the effect of sodium chlorate on Canada thistle growing in grassland. A, thistle from control plot; B, thistle from plot treated with 150 kgm.; and, C, thistle from plot treated with 200 kgm. per hectare, on November 11, 1925. On plots treated with larger quantities no thistles appeared the following year. Thistles harvested and photographed on June 30, 1926

oats as well as the thistles. The perchlorate, in amounts of 400 p. p. m. or more, killed the thistles and oats in 40 days. Other herbicides applied had no appreciable effect on the plants.

SECOND FIELD EXPERIMENT

THE APPLICATION OF HERBICIDES AT VARIOUS SEASONS

In the first field experiment it was found that chlorates applied on the ground late in autumn eradicated Canada thistle before the following spring, and in pot experiments sodium cyanide and thiocyanate were found more poisonous to the thistle than the chlorates. In order to compare these herbicides under field conditions, the following experiment was conducted.

In June, 1926, a field on the farm of the New York State College of Agriculture, Ithaca, N. Y., was secured for the experiment. This

field had been sown to oats and was heavily infested with Canada thistles. The soil was of the Dunkirk silty clay-loam type, rather poor in organic matter and with a reaction of P_H 5.3. The subsoil was very compact and had poor drainage. Plots 10 square meters in area were laid out. A diagram of these plots is shown in Figure 2. On June 22 the number of thistles was determined on each plot. The results are recorded in Tables 1 to 4. It was decided to apply the herbicides not only in different amounts but also at different seasons. The first application was made on June 28; the second on September 1, just after the oats were harvested; the third on November 1; and the last on March 30 the following spring. Only one plot for each treatment was possible, as five different amounts of each herbicide were used at each time and the field was of limited area. The herbicides were applied dry and broadcast as evenly as possible. The following commercial products were used: Sodium chlorate, $NaClO_3$; potassium chlorate, $KClO_3$; sodium cyanide, NaCN; and sodium thiocyanate, NaCNS. The quantities employed were from 100 to 300 kgm. per hectare. The applications were made in accordance with a certain scheme which distributed the treatments evenly over the experimental field. One-sixth of the plots were left untreated. The amounts applied to each plot are recorded in Tables 1 to 4. At intervals the effect of the herbicides on the vegetation was noted.

1	2	3	4	5	6	7	8	9	10	11	12
13	14	15	16	17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32	33	34	35	36
37	38	39	40	41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70	71	72
73	74	75	76	77	78	79	80	81	82	83	84
85	86	87	88	89	90	91	92	93	94	95	96

FIG. 2.—Diagram of plots in second field experiment. The plots were nearly square and were separated by paths 1 meter wide.

TABLE 1.—*Number of shoots of Canada thistle on plots 10 square meters before and after treatment with herbicides, and yield of oats in kilograms per hectare; herbicides applied June 28, 1926*

Plot No.	Treatment (kgm. per hectare)	Number of thistle shoots		Yield of oats, Aug. 13, 1927 (kgm. per hectare)	
		June 22, 1926	Aug. 13, 1927	Grain	Straw
	Na CN				
24	100	112	56	1,250	1,320
14	150	84	77	1,520	1,600
9	200	198	162	1,320	1,580
4	250	101	15	1,380	1,380
19	300	82	92	1,290	1,330
	$KClO_3$				
2	100	7	15	1,940	2,200
22	150	159	5	1,240	1,220
17	200	128	7	1,270	1,260
12	250	120	0	1,360	1,210
7	300	164	2	1,200	1,100
	$NaClO_3$				
8	100	140	19	1,640	1,930
3	150	43	1	1,240	1,250
23	200	157	0	1,310	1,230
18	250	82	1	1,040	900
13	300	49	0	1,270	1,420
Controls untreated (average)	----------	124	108	1450±126	1670±142

TABLE 2.—*Number of shoots of Canada thistle on plots 10 square meters before and after treatment with herbicides, and yield of oats in kilograms per hectare; herbicides applied September 1, 1926*

Plot No.	Treatment (kgm. per hectare)	Number of thistle shoots		Yield of oats, Aug. 13, 1927 (kgm. per hectare)	
		June 22, 1926	Aug. 13, 1927	Grain	Straw
	Na CN				
27	100	92	191	1,910	2,830
31	150	87	141	1,600	1,920
20	200	131	160	1,220	1,720
11	250	132	201	1,000	1,520
34	300	94	162	1,460	1,830
	Na CNS				
35	[a] 100	101	96	1,200	1,600
36	[a] 150	44	43	1,390	1,710
37	[a] 200	172	98	1,420	1,930
39	[a] 250	158	108	2,230	3,160
42	[a] 300	160	84	1,640	3,210
	K ClO₃				
5	100	178	32	1,360	1,480
28	150	15	12	1,660	2,170
25	200	120	2	1,310	1,460
15	250	48	18	1,240	1,200
32	300	172	0	850	760
	Na ClO₃				
16	100	59	38	1,080	1,040
10	150	162	39	1,020	1,000
30	200	141	0	1,240	1,320
26	250	162	1	1,520	1,900
33	300	168	0	560	630
Controls untreated (average)		105	83	1,510±112	1,730±126

[a] Applied September 25.

TABLE 3.—*Number of shoots of Canada thistle on plots 10 square meters before and after treatment with herbicides, and yield of oats in kilograms per hectare; herbicides applied November 1, 1926*

Plot No.	Treatment (kgm. per hectare)	Number of thistle shoots		Yield of oats, Aug. 13, 1927 (kgm. per hectare)	
		June 22, 1926	Aug. 13, 1927	Grain	Straw
	Na CN				
43	100	121	111	1,740	2,100
62	150	162	140	1,500	[a] 2,800
58	200	106	118	1,570	2,230
51	250	132	201	1,700	2,480
47	300	48	96	1,490	1,920
	Na CNS				
56	100	111	152	2,080	[a] 3,500
50	150	140	182	1,780	[a] 3,660
46	200	102	71	1,440	2,180
67	250	104	86	1,460	2,000
61	300	112	121	1,480	2,600
	K ClO₃				
44	100	113	56	1,700	1,940
63	150	90	92	1,540	1,770
59	200	43	6	1,400	1,760
54	250	148	1	1,360	1,520
48	300	28	4	1,350	1,520
	Na ClO₃				
49	100	151	68	1,400	[a] 2,400
45	150	190	6	1,160	1,160
66	200	64	0	1,260	1,040
60	250	28	1	1,200	1,160
55	300	86	0	1,300	1,040
Controls untreated (average)		71	142	1,360±86	[a] 2,650±83

[a] Straw yields include thistles.

TABLE 4.—*Number of shoots of Canada thistle on plots 10 square meters, before and after treatment with herbicides, and yield of oats in kilograms per hectare; herbicides applied March 30, 1927*

Plot No.	Treatment (kgm. per hectare)	Number of thistle shoots		Yield of oats, Aug. 13, 1927 (kgm. per hectare)	
		June 22, 1926	Aug. 13, 1927	Grain	Straw
	Na CN				
73	100	33	61	1,320	2,000
93	150	73	42	890	700
87	200	5	43	1,520	* 2,370
83	250	39	26	1,080	1,320
79	300	99	87	1,940	2,380
	Na CNS				
91	100	64	64	1,370	1,500
86	150	6	72	1,750	* 2,700
82	200	81	48	460	520
77	250	68	78	200	340
96	300	5	20	0	0
	K ClO$_3$				
80	100	61	78	0	0
75	150	0	17	0	0
94	200	100	12	0	0
89	250	62	52	0	0
84	300	13	0	0	0
	Na ClO$_3$				
85	100	60	65	410	380
81	150	76	6	0	0
76	200	13	4	0	0
95	250	64	7	0	0
90	300	39	15	0	0
Controls untreated (average)		29	62	1,310±57	1,830±73

* Straw yields include thistles.

Cyanide applied on June 28 rapidly killed the surface vegetation of the plots. New thistle shoots appeared soon afterwards, indicating that the roots were unharmed. The application made on September 1, or just after the oats were harvested, did not injure the vegetation. Seedlings of oats, ragweed, *Ambrosia artemisifolia* L., yellow foxtail, *Setaria glauca* (L.) Beauv., and shoots of Canada thistle grew vigorously during the autumn, indicating that the nitrogen of the cyanide had been available as plot nutrients. The application on November 1 killed the vegetation within a fortnight.

The thiocyanate was not applied before September 25. Its application at that date had a marked influence on the vegetation. The larger amounts killed the annual vegetation and harmed the thistles considerably. Similar effects were noted after the application on November 1.

The effect of the chlorates applied in June was not very noticeable. The oats continued to grow without injury and the thistles became only a little scorched. The effect of the potassium chlorate was less apparent than that of the sodium chlorate, probably owing to lower solubility. This inhibition of the effect of the chlorates may be explained as due to lack of rain. The salts did not dissolve, or at least did not penetrate to the absorbing parts of the roots of the plants. However, when the oats were harvested, and the new vegetation appeared on the untreated plots, the chlorate plots remained bare of annual plants. The poisons prevented seeds from germinating or

killed the seedlings. The thistles were less injured. Some leaves were scorched, but in other ways they were growing almost as well as on the untreated plots. The application of chlorates on September 1 had a similar effect on the vegetation, for rain dissolved the salts rather soon. The chlorates applied on November 1 killed the annual plants in about three weeks, but the thistles were not seriously harmed. There was no difference in the action of potassium and sodium chlorate.

The field was not plowed in the autumn. On May 7, the plots were disked three times, fertilizers broadcast over the field, and Early Dakota oats sown. The cold weather during May checked the growth, so that the oats were rather weak when the drought in June set in. As a result the crop yields were rather low. The oats were harvested on August 13 and threshed. The results, which are recorded in Tables 1 to 4, show that the yields vary considerably. The probable error of untreated plots is large. There is a clear detrimental effect of chlorates and thiocyanate applications that were made in the spring, five weeks before the oats were sown. Thiocyanate applied the preceding year had no effect on the yield of oats, while the highest applications of chlorates in some instances decreased the yields. The decrease in yields of straw is, however, in most instances due to decrease in number of thistles. The sodium cyanide in several cases shows a distinct fertilizing effect.

The number of thistles on the plots were counted several times during the summer of 1927; the numbers found on August 13, when the plots were harvested, are recorded in Tables 1 to 4. On the cyanide and thiocyanate plots there was no influence of the herbicides on the thistles. On the chlorate plots, on the contrary, the thistle shoots failed to appear or were found late in the season.

EXPERIMENT TO DETERMINE PENETRATION OF HERBICIDES THROUGH SOIL

Of the various herbicides used in the first and second field experiments only the chlorates had eradicated the thistles. The preliminary pot experiments had shown sodium cyanide and thiocyanate to be more toxic than chlorates to the Canada thistle. Work reported in literature shows that soluble arsenite had no effect on the Canada thistle. As the herbicides were applied on the surface of the ground, while the horizontal "propagation roots" of the thistle were found at a depth of 15 to 20 cm., it is clear that the herbicides must penetrate the soil to that depth in order to affect the plants. The difference in action between the herbicides tested may for that reason be due partly or wholly to different rates of penetration through the soil in the field as compared with soil in pots. Some experiments were performed in order to determine if the herbicides penetrate a soil at different rates and if penetration is influenced by soil type. The penetration of solutions of herbicides through columns of soil was determined. However, in order to be sure that the values obtained represented the penetration of the herbicides through soil special cylinders were made for the soil columns.

Apparatus Used

If the penetration of a solution through soil is measured when the soil is kept in containers such as cylinders of glass, metal, etc., the values obtained may be influenced by the nature of the cylinder. A

solution may flow more easily along the wall of the cylinder than through the soil. Absorption or adsorption of solution by the wall of the cylinder may influence the results. In order to avoid these sources of error cylinders were made of fine mesh wire netting and coated with paraffin as follows: Cylinders were made of wire netting with meshes about 3 mm. 35 cm. in height, and with a cross section area of 1 sq. decim. A bottom of the same netting was inserted 5 cm. from the lower end and the edges of the cylinder soldered together. Thereafter the cylinder was dipped in melted paraffin until a thin coating was formed. A filter paper was placed in the bottom of the cylinder and dry soil, passed through a sieve (20 meshes per inch), was added with uniform packing to a height of 15 cm. The paraffin coating the part of the cylinder that was filled with soil was then melted. The melting paraffin was absorbed by the soil. The cylinder, while still hot, was dipped several times in melted paraffin until a rather thick coating was formed. Enough water was then added to saturate the soil, and the cylinder was left standing until the next morning, when the water was found to have reached the bottom of the soil column. The paraffin on the bottom of the cylinder as well as the filter paper was now perforated at every mesh of the netting, so that the water could flow through. The wet soil did not pass through these holes.

Two sets of cylinders were made. The first set was filled with Dunkirk silty clay loam collected from the field on which the second field experiment was located. The reaction of the soil was P_H 5.77 as measured by the quinhydrone electrode. As the reaction of the soil might have some influence on the penetration of the herbicides, a second set of cylinders was made and filled with Lansing silt loam which had a reaction of P_H 7.87. Each set was made up of four cylinders as the penetration of four herbicides—namely, sodium chlorate, $NaClO_3$; sodium thiocyanate, NaCNS; sodium cyanide, NaCn; and sodium arsenite, $NaHAsO_3$—was to be determined. The cylinders were mounted on Büchner funnels, filled with water, and left for three days in order that the soil might become saturated. The penetration of water was measured and compared with the penetration of the herbicidal solutions later on.

At the outset of the experiment, the cylinders were emptied of water and 1 liter of N/10 solutions of the herbicides was added. The amount of solution in the cylinders was kept constant by daily addition of amounts equal to those leaching through the soil. The amount of solution leaching through the soil was recorded daily and analyzed for the herbicides.

Analytical Methods

The analysis for chlorates was performed according to the method of Treadwell and Hall (*32*). A sample of the solution to be analyzed was transferred to an Erlenmeyer flask fitted with a Bunsen valve. The air was expelled with a stream of carbon dioxide. A definite amount of an acid iron sulphate ($FeSO_4$) solution was added, and the mixture boiled. Chlorates oxidize the iron sulphate to $Fe_2(SO_4)_3$ and the excess of $FeSO_4$ was determined by titration with a potassium permanganate solution.

Analyses for thiocyanate were made colorimetrically. To a given amount of the solution (leaching), 24 c. c. in the first tests and 1 c. c. in the last, 1 c. c. of a normal $Fe_2(SO_4)_3$ solution was added. The

amount was made up to 25 c. c., filtered, and the color compared with a standard made up of 24 c. c. N/500 NaCNS plus 1 c. c. $NFe_2(SO_4)_3$ solution. A Duboscq colorimeter was used.

The analyses for cyanide were made according to the Official Methods (*7*). Ten cubic centimeter portions of the solution were titrated with a N/50 $AgNO_3$ solution until a faint turbidity was noticed.

The analyses for arsenic were made according to the method used by Schulz and Thompson (*28*). The essential feature of the analysis is that the evolving arsine is caught in a 5 per cent solution of mercuric chloride ($HgCl_2$) and determined by titration with sodium thiosulphate ($Na_2S_2O_3$).

Results

Chlorates appeared in the solution leaching through the Dunkirk silty clay loam after five days, and in the leachings from the Lansing silt loam after three days. The thiocyanate was found in leachings from the former soil after four days and in leachings from the latter soil after three days. Cyanides were not found to penetrate either soil. Analyses were made of leachings from Dunkirk soil for 40 days and for the Lansing soil for 30 days. Arsenic was found in a concentration of N/1000 in leachings from the Dunkirk soil after 34 days, increasing to about N/500 10 days later. The solution from the Lansing soil gave tests for arsenic after 18 days; on the thirtieth day the concentration in the leachings was N/400.

The chlorate and thiocyanate had no noticeable influence on the soil in the cylinders. The amount of solution leaching through remained constant and was uncolored throughout the experiment. The cyanide and arsenite solutions had a dissolving action on the organic matter of the soil. The supernatant liquid in the cylinders became brown and even the solutions leaching through soon were of a brownish color. These solutions also made the soils less permeable. The amount of solution leaching through the soil columns decreased to about one-third in 21 days, the period during which the leachings were measured daily.

The curves in Figure 3 illustrate the leaching of sodium chlorate and sodium thiocyanate through Dunkirk silty clay loam. It is seen that they penetrate freely. After 18 days the concentration of the solution leaching through a soil column of 15 cm. was found to be equal to the concentration of the solution added. The similarity of the curves indicates that the salts probably do not react with any substances in the soil.

A solution of sodium cyanide is very alkaline. Its slow penetration is probably, in part at least, due to this fact. When the above experiments were concluded, the penetration of a solution of sodium cyanide which was titrated to neutrality was determined. It was found to leach through the Dunkirk soil column in 22 days. The amounts were very small.

Experiment to Determine Decomposition of Herbicides in the Soil

A preliminary experiment showed that sodium cyanide and thiocyanate disappear rather soon from soil kept in a greenhouse. If there is a rapid disappearance it may prevent their action in field experiments. However, the decomposition may be assumed to proceed more rapidly in soil kept in a greenhouse of high temperature and at optimum moisture content than in a field. If the temperature

is found to play an important part in the decomposition of the herbicides their application late in autumn or early in spring on ground of low temperature may give the desired results.

The following experiment was conducted in order to study the influence of temperature on the decomposition of the herbicides. Ordinary glass tumblers were filled with 200 gm. of moist soil collected in a field in the beginning of April. This moist field soil was preferred to the dry greenhouse soil as the catalytic power of the soil in some way might influence the decomposition of the herbicides. The catalytic power is known to diminish very markedly when the soil is dried. The moisture content of the soil was 24.9 per cent; so that each tumbler contained 160.2 gm. of dry soil. The tumblers were covered with Petri dishes and placed in the dark in incubators at 10°, 20°, and 30° C. A week later 10 c. c. of a solution containing 16 mgm. of herbicide was added to each tumbler, thus making the concentration in the tumbler 100 p.p.m. of dry soil. The solutions were well mixed with the soil. Sodium chlorate, sodium thiocyanate, and sodium cyanide were the herbicides applied. Fifteen tumblers were left untreated for controls.

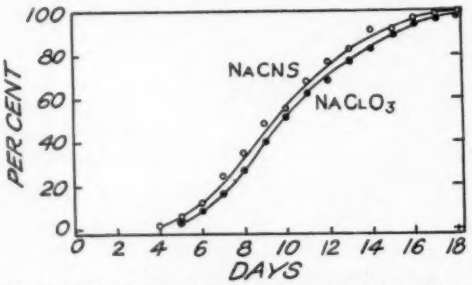

FIG. 3.—Penetration of sodium thiocyanate and sodium chlorate through Dunkirk silty clay loam. After 18 days the concentration of the solution leaching through a 15 cm. soil column was equal to the concentration of the solution added

One tumbler of each series was planted each week for 10 weeks. As the chlorates were expected to decompose more slowly than the other herbicides, especially at lower temperature, 15 tumblers containing that herbicide were kept at 10° C. Controls from the three temperatures used were planted every other week. As the experimental plant Alpha barley was used. Ten seeds were planted and five seedlings allowed to grow in each tumbler. The seeds were planted in the first series of tumblers on April 9, 1927, one week after the herbicides were applied. After the seeds were planted, the tumblers were kept in the incubators until the seedlings were 3 to 4 cm. high. The tumblers were then moved to a greenhouse. Notes on the height and appearance of the plants were taken on removal to the greenhouse and thereafter at weekly intervals.

It was found that sodium cyanide decomposed so rapidly even at low temperatures, that the plants grew better in tumblers treated with that herbicide than in the controls. The thiocyanate decomposed so rapidly in tumblers kept at 20° and 30° C. that it had no poisonous effect on the plants. At 10° this herbicide did not decompose rapidly enough to leave unaffected the first barley planted. The plants sown one week after the poison was applied died at an average height of 4.5 cm. Plants sown one week later attained an average height of 12 cm. Plants from seeds sown later were somewhat stunted in the early stages of growth but recovered and grew to full size. The sodium chlorate had a much more lasting effect. In tumblers kept at 10° the barley seedlings died rapidly. The plants

from the first sown barley reached an average height of 2 to 3 cm.; barley sown up to 15 weeks after the herbicide was applied reached a height of 8 to 10 cm. Plants in the tumblers kept at 20° attained a larger size before dying, especially in those sown later. The difference was still more marked in tumblers kept at 30°. In these the seedlings grew to about 10 cm. in the first sown tumblers, and in the one sown 10 weeks after the herbicide was applied the plants seemed to grow to full size. When the uninjured plants had reached a height of about 30 cm. they were discarded. The difference in the size of the plants reached before death probably did not give a true indication of the decomposition of the chlorate. In the tumblers kept at the higher temperatures the seedlings grew very fast, so that they had reached a larger size before the herbicide had had time to act. The time necessary for the chlorate to kill the seedlings was about equal for all temperatures in the beginning of the experiment. The seedlings died after growing for three weeks in the greenhouse. In the latter part of the experiment, however, the difference in time was striking. In tumblers kept at 10° the plants died almost as soon as in the beginning of the experiment, or in about three weeks in the greenhouse, while in the 20° tumblers they were growing for six weeks in the last-planted tumbler, and in the tumbler kept at 30° for ten weeks they seemed to grow indefinitely. Thus the action of the chlorate diminished with time, especially at higher temperatures. This seems to have been due to the decomposition of the chlorate as no leakage was possible.

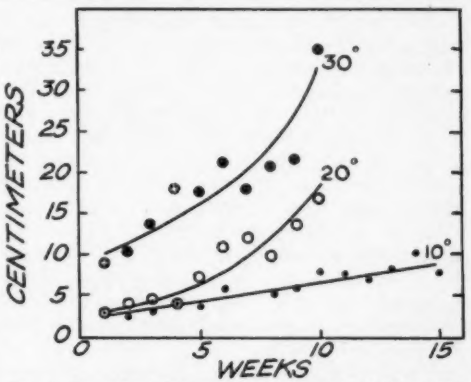

FIG. 4.—Decomposition of sodium chlorate at 10°, 20°, and 30° C. Determined by the height (cm.) attained by barley seedlings when grown in soil to which was added 100 p. p. m. of sodium chlorate

In Table 5 and in Figure 4 the main effects of sodium chlorate upon barley seedlings are shown. The effect of sodium cyanide and thiocyanate are not given as these herbicides disappeared so rapidly.

TABLE 5.—*Average height attained by barley seedlings planted in soil to which was added 100 p. p. m. of sodium chlorate and kept at different temperatures for increasing lengths of time before planting*

Number of weeks interval between application of herbicide and planting of barley seed	Average height of seedlings kept at—			Number of weeks interval between application of herbicide and planting of barley seed	Average height of seedlings kept at—		
	10° C.	20° C.	30° C.		10° C.	20° C.	30° C.
	Cm.	*Cm.*	*Cm.*		*Cm.*	*Cm.*	*Cm.*
1	3.0	3.0	9.0	9	6.0	14.0	22.0
2	2.6	4.0	10.4	10	8.0	17.0	35.0
3	3.0	4.5	14.0	11	7.6	------	------
4	4.0	4.0	18.0	12	7.0	------	------
5	3.6	7.6	17.6	13	8.0	------	------
6	6.0	11.0	21.7	14	10.0	------	------
7	------	12.0	18.0	15	9.0	------	------
8	5.0	10.0	21.0				

EXPERIMENTS TO DETERMINE THE EFFECT OF HERBICIDES ON BIOLOGICAL ACTIVITIES IN THE SOIL

The importance of the biological processes occurring in the soil can not be overestimated. Any operation that disturbs the biological balance in a soil will influence its fertility. Partial sterilization of the soil has often increased the fertility, the reason for which has been interpreted in various ways. The application of herbicides in amounts sufficient to eradicate Canada thistle may be suspected to have some influence on the biological activities of the soil. An investigation was made of the effect of the herbicides used in the second field experiment upon the ammonification and nitrification processes, soil protozoa, and earthworms. A short summary of the results is given below.

Effect on Ammonification and Nitrification

Two series of experiments were conducted.

1. The accumulation of ammonia and nitrates was determined in soil samples that were collected on the plots of the second field experiment in the spring of 1927. It was found that the herbicides had no effect on the ammonification process. The accumulation of nitrates was also unaffected by the application of herbicides in the autumn.

2. Definite amounts of herbicides, from 50 to 2,000 p. p. m., were added to soil samples, and the accumulation of ammonia and of nitrates was determined. It was found that sodium chlorate and thiocyanate had no influence on the ammonification process, while cyanide had a retarding effect. The accumulation of nitrates was diminished by all herbicides. Thiocyanate applied at the rate of 500 p. p. m. not only inhibited nitrate accumulation but reduced the amount of nitrate originally present in the soil. The retarding effect of cyanide was less and sodium chlorate had the least influence on the process.

Under field conditions the herbicides should be applied in the autumn. The experiments show that an application at that time has no influence on the ammonification and nitrification processes the following spring.

Effect on Soil Protozoa

Herbicides were added to soil samples at the rate of 50 to 2,000 p. p. m. After 10 days samples of the cultures were transferred to sterile media and after 10 days of incubation the developing protozoa were studied. The results indicate that sodium chlorate had no influence on the number of protozoa, thiocyanate had a doubtful action, and cyanide had a harmful effect. Later on it was found that protozoa were alive for five days in N/100 solutions of the herbicides. The sodium cyanide solution used in that test was titrated to neutrality. The protozoa seemed very sensitive to the alkaline reaction of an untitrated solution. Thus it seems that at least some of the protozoa are not injured by the herbicides.

Effect on Earthworms

Earthworms were placed in soil to which herbicides were applied at the rate of 100 to 2,000 p. p. m. After 10 days those surviving were counted. Chlorates produced little or no injury. This result is sup-

ported by observations on experimental plots. The highest amount of thiocyanate killed the worms, and of cyanide even 100 p. p. m. was fatal.

DISCUSSION

DISAPPEARANCE OF THE HERBICIDES IN THE SOIL

Disappearance by Leaching

The action of the herbicides under field conditions was of comparatively short duration. Oats grew well on plots that were treated with enough of a herbicide to eradicate Canada thistle if the application was made in the autumn of the previous year. The disappearance of the herbicides may have been due to leaching or decomposition or both.

The curves in Figure 3 show that $NaClO_3$ and NaCNS penetrate the soil easily. It is clear that the disappearance of these herbicides may, in part at least, be due to leaching. On the contrary, NaCN and $NaHAsO_3$ penetrated the soil but slowly or not at all. Any appreciable leaching of these herbicides is thus not to be expected on heavy soil. In fact, McGeorge (*23*) found no leaching of arsenic from the surface soil on which plants had been treated with arsenical sprays. Schulz and Thompson (*28*) found that the greater part of the arsenite applied to a barberry bush had leached out after one year. The soil on which the bush was growing is described as "well drained soil, rich in leaf mold."

Disappearance by Decomposition

DECOMPOSITION OF SODIUM CYANIDE

The experiments show that the decompostion of the herbicides varied for each herbicide used. NaCN decomposed very rapidly under "natural" conditions, i. e., in soil of optimum moisture content for plant growth. When NaCN was added, in amounts of 100 p. p. m. of dry soil, to growing plants it killed them overnight or within a few days. If the application was made at the same time that the barley seed was planted the seedlings grew better in the treated soil than in the untreated controls. Petit (*26*) also has found that small amounts of KCN and other cyanogen compounds increased the yield of some plants in pot cultures. Brenchley (*8*) and Hawkins (*17*) found HCN or KCN to be very toxic to plants grown in solution cultures. Clark (*9*) found these substances very toxic to fungi.

The fact that NaCN decomposes rapidly in soil of optimum moisture content which has a very alkaline reaction suggests that its decomposition is brought about by microorganisms. Results reported by Gardner (*14*), Robbins (*27*), and others show that a number of organic substances toxic to higher plants are decomposed by microorganisms.

When cyanides decompose ammonium formate is formed. This compound after proper acidation is probably easily utilized by plants, as is indicated by the beneficial effect of decomposed cyanide. The rapid decomposition of NaCN explains why it had no effect on the Canada thistle under field conditions. Its action is reduced still more because it does not easily penetrate the soil.

DECOMPOSITION OF SODIUM THIOCYANATE

In soil kept at 10° C. an amount of 100 p. p. m. killed barley seedlings from seed planted two weeks after the poison was applied. Barley planted later survived. In soil kept at higher temperatures the poison disappeared earlier. That the decomposition of thiocyanate is not always so rapid is indicated by the action of the thiocyanate in the second field experiment. An application of 250 kgm. NaCNS or more per hectare on March 30 inhibited the growth of oats sown on May 7, or five weeks later. In spite of a rather slow decomposition under wet and cool conditions and rapid penetration through soil, for reasons which are not quite clear, NaCNS failed to eradicate Canada thistles under field conditions.

DECOMPOSITION OF CHLORATES

Chlorates were found to decompose rather slowly. However, that they decompose is clear. Their disappearance under field conditions is not wholly due to leaching. In soil kept at 30° C., $NaClO_3$ decomposed markedly during 10 weeks. Barley planted 10 weeks after the herbicide was applied grew to full size while that planted earlier died. In soil kept at a lower temperature the decomposition was slower, but noticeable.

The decomposition is probably due to microorganisms. Alvisi and Orabona (*2*) found that *Penicillium glaucum* Link decomposed $KClO_3$ and NH_4ClO_3. Virgili (*33*) found that chlorates disappeared in 63 days in meat allowed to putrefy. The writer found that species of Penicillum, Aspergillus, and Fusarium grew on top of hay infusions containing N/10 $NaClO_3$ solution. Numerous bacteria were also observed growing in the solution. Such a solution was found to decompose slowly. For instance, after standing for 60 days with only a very small surface exposed to the air the strength was reduced from N/10 to about N/15. The decomposition thus seems to take place under more or less anaerobic conditions. The decomposition is a reduction by which free oxygen is given off. Its occurrence under anaerobic conditions seems rather natural.

It was observed that the decomposition takes place in soil supersaturated with water. Dunkirk silty clay loam used in the penetration experiments did not give a test for chlorides. However, after a N/10 $NaClO_2$ solution had leached through for 30 days the leachings gave a good test for chlorides. The chlorate used in the experiment was free from chlorides, so that the chlorides in the penetrating solution appear to be reduced chlorates. The slow decomposition of the chlorates and the ease with which they penetrate a soil explains their destructive effect on the Canada thistle.

DECOMPOSITION OF ARSENITE

Of the herbicides used in the experiments, sodium cyanide, sodium thiocyanate, and sodium chlorate are compounds of nonpoisonous elements. It is the peculiar combination that makes these compounds toxic. When they are decomposed the constituents are harmless. On the contrary, the toxic element in arsenite is its content of arsenic. It is clear that decomposition of the arsenite does not destroy the poisonous properties of the arsenic. The toxicity may diminish if the

new compound is less soluble, but in solution it is proportional to the arsenic content.

Arsenic combines easily with other elements, so that it is probable that arsenite used as a herbicide decomposes rather rapidly in the soil. As the arsenic is the carrier of the toxic properties it is clear that a soil treated with arsenic can be freed from the poison only by leaching. The experiments show that sodium arsenite penetrates a soil very slowly. Thus a soil treated with appreciable amounts of arsenite remains unfit for plant growth for a long time. Gray (*16*) reports that plots treated with arsenical spray for the eradication of morning glory were bare of all vegetation for 14 months.

THE USE OF HERBICIDES ON ARABLE LAND

The eradication of perennial weeds is an expensive operation. One of the best methods for this purpose is the use of a proper summer fallow. Where labor is inexpensive, the thorough cultivation brought about by the summer fallow is probably the best method for eradication of perennial weeds on arable land. However, the cost of labor may increase and lighter soil may not benefit from the summer fallow tillage as heavy soils have been shown to do. Moreover, fallowing is an operation that is suited only for large areas. Perennial weeds often appear in colonies, and their eradication on smaller areas before they have a chance to spread over the field is desirable.

Chlorates, and especially sodium chlorate, gave the best results of the herbicides tested on Canada thistle in these experiments. It was easily applied as dry salt; it penetrated the soil easily, so that it reached the roots of Canada thistle rapidly; it decomposed comparatively slowly, so that it acted efficiently; it could be applied with little or no interference with crop production; it was found to have but slight influence on the biological activities in the soil.

On very heavy soil the chlorates may be assumed to penetrate too slowly. It was found in the second field experiment that an application of more than 200 kgm. of sodium chlorate per hectare in some cases decreased the yield of oats sown the following spring. The herbicide had not disappeared rapidly enough. The horizontal propagation roots of Canada thistle grow at a depth of 15 to 30 cm. The best time for the application of chlorates was found to be in the autumn after the crop was removed. If the weedy field is to be fall plowed, an application of this herbicide in the furrows by some attachment to the plow would facilitate its action. In this way the herbicide would come in contact with the roots without necessitating its penetration through the first 15 to 20 cm. of soil. Smaller amounts than those used in these experiments may be found sufficient if this scheme is followed.

Chlorates applied in autumn were found to be very efficient for the eradication of Canada thistle. Several other perennial weeds, having less easily affected roots, or rootstocks, are more difficult to eradicate by chlorates. Korsmo (*19*) found an application of sodium chlorate, 500 kgm. per hectare, insufficient to eradicate quack grass. Fron and Arnal (*13*) found an application of 2,000 kgm. per hectare necessary for the eradication of *Spartium junceum* L., a leguminous shrub. In both cases the application was made in the spring, which may have diminished the action of the herbicide.

SUMMARY

The purpose of the experiments here reported was to develop a method for the eradication of Canada thistle, *Cirsium arvense* (L.) Scop., from arable land by the use of herbicides. The additional effects of the herbicides upon the soil were also determined.

Sodium chlorate, $NaClO_3$; potassium chlorate, $KClO_3$; sodium thiocyanate, NaCNS; sodium cyanide, NaCN; and sodium arsenite, $NaHAsO_3$ were the herbicides used.

An application of 200 kgm. per hectare of sodium chlorate (or 250 kgm. potassium chlorate) per hectare as dry salt on the ground late in the autumn killed the roots of Canada thistle during the winter. An application early in the spring was less effective. Other herbicides used had practically no effect on the Canada thistle under field conditions.

The effectiveness of chlorates is due to their rapid penetration through soil and their slow decomposition, especially at low temperatures. Sodium thiocyanate, and to a greater extent sodium cyanide, decomposed so rapidly in the soil that no harm was done to Canada thistle under field conditions. Sodium cyanide did not seem to penetrate the soil under field conditions. Sodium arsenite was ineffective against Canada thistle because it penetrated the soil very slowly. A special apparatus was constructed for determining the rate at which the herbicides penetrated the soil.

An application of herbicides in the autumn had no influence on the ammonification and nitrification processes in the soil the following spring.

An application of 200 kgm. per hectare of sodium chlorate in late autumn killed the Canada thistles and did not injure the oats that were sown on the plots the following spring.

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DOWNY MILDEW (SCLEROSPORA GRAMINICOLA) ON EVERGLADE MILLET IN FLORIDA[1]

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INTRODUCTION

Among the downy mildews or Peronosporaceae, the genus Sclerospora has attracted increasing attention on account of its destructive parasitism of various valuable gramineous crops. Of this genus, the species *Sclerospora graminicola* (Sacc.) Schroet. is the most widely distributed, occurring in northern temperate portions of the United States and Europe and in both temperate and tropical regions of Asia, and causing in the aggregate considerable injury to millet and related crops. Yet the present knowledge of this fungus in many respects is scanty, and additional information as to its occurrence in new localities or on additional hosts, its severity, persistence, and spread under different conditions, is necessarily of interest from both the agricultural and mycologic points of view.

Therefore, when this species, for the first time in this State and on this host, was found by the junior writer in November, 1922, on Everglade millet at Vero Beach, Fla., and when during the following year it was encountered also in other widely separated parts of the State, it seemed important to investigate the destructive activity of the parasite on this new host under subtropical conditions and to appraise the possibility of its becoming a menace to Florida's agriculture. The results of this investigation are presented in the following paper.

THE DISEASE

OCCURRENCE IN FLORIDA

Ever since the first discovery of the fungus in Florida an intermittent but extensive search has been made to determine on what other hosts and in what other parts of the State it might occur. Up to the present, however, only the one species of host has been found to be infected under natural conditions. This grass, the Everglade millet,[3] *Chaetochloa magna* (Griseb.) Scribner (*Setaria magna* Griseb.), is very common throughout the State in low marshy ground

[1] Received for publication Feb. 2, 1928; issued August, 1928.

[2] The senior writer wishes to express his thanks to the Office of Cereal Crops and Diseases for supporting his share of these investigations in Florida in June, 1924, while on leave from Harvard University, and to Hugh M. Matheson, of Miami, Fla., for courtesies which facilitated the intensive study of the mildew. He wishes also to acknowledge his indebtedness for aid from the Milton Research Fund, of Harvard University, which expedited the long-delayed preparation of the manuscript and plates by relieving him of some of the demands of academic work.

[3] The writers take this opportunity of thanking Mrs. Agnes Chase for her kindness in verifying the identification of the host.

on muck or peat soils, either quite outside of cultivation or as a weed in poorly cultivated fields. In the monograph of Scribner and Merrill (*14*, *p. 21*) [4] the grass is described as "a coarse, stout, erect perennial (?) 10 to 36 dm. high, with cylindrical culms 0.5 to 2 cm. thick at the base, linear-lanceolate leaves and dense, cylindrical panicles 1.5 to 3 dm. long, * * * from Delaware to Florida, Louisiana, and western Texas." Of its perennial character there may be doubt, as indicated by the question mark, in the northern

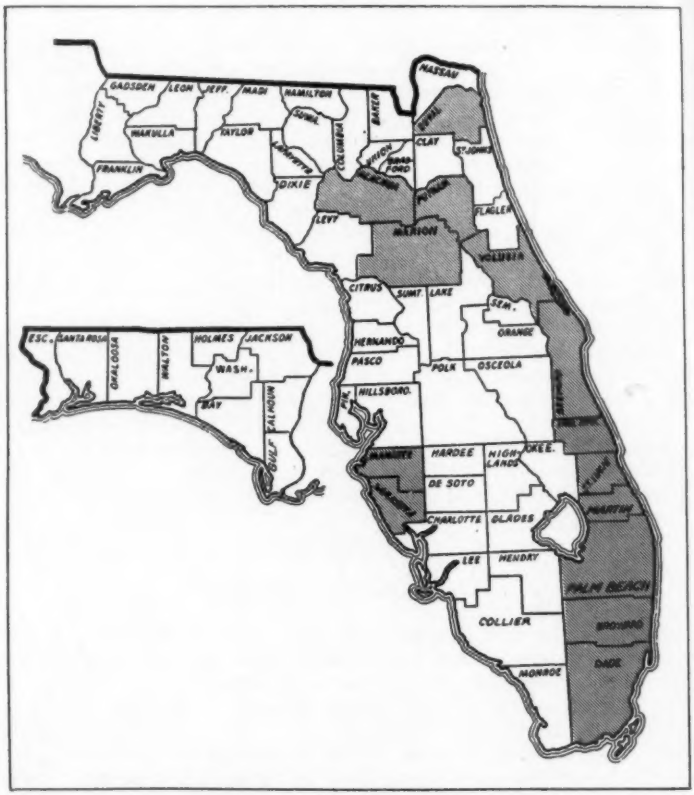


FIG. 1.—Sketch map of Florida. Shaded areas show counties in which *Sclerospora graminicola* has been collected on Everglade millet

limits of its range; but in Florida, at least, it is indeed perennial, forming persistent, heavy clumps that continually put forth new growths.

In Florida, as noted by the writers in a preliminary report (*22*), the fungus invariably has been found wherever this grass was encountered, the distribution of the parasite and of the host apparently being coincident. So far they have been collected from various parts

[4] Reference is made by number (italic) to "Literature cited," p. 962.

of the following counties: Dade, Broward, Palm Beach, Martin, St. Lucie, Indian River, Brevard, Volusia, Duval, Putnam, Alachua, Marion, Manatee, and Sarasota. As is obvious from the accompanying map (fig. 1), these localities cover representative regions of the whole State from the more northern portion that resembles adjacent territory of sister States to the subtropical peninsula which is peculiar to Florida.

This occurrence of *Sclerospora graminicola* on *Chaetochloa magna* in Florida is an interesting addition to our knowledge of the host range and the geographic distribution of the fungus. Since the species was described first by Schroeter (*13*) in 1879 from Baden, Germany, on *Setaria* (*Chaetochloa*) *viridis* Beauv., it has been reported most commonly throughout Europe on this host from various localities in Italy, France, Germany, Czechoslovakia, and Russia, while on the two closely related wild grasses *C. glauca* (L.) Scribn. and *C. verticillata* (L.) Scribn. it has been found also with a very similar distribution. On the cultivated Italian millet, *C. italica* (L.) Scribn., it has been reported not only throughout Europe but also from points in India, China, and Japan. On *Pennisetum typhoideum* Rich. it has been recorded from German East Africa and from two widely separated points in India. It is of interest that all of these hosts are in the tribe Paniceae of the family Gramineae. As to the occurrence of this fungus on hosts outside of this tribe, there is the somewhat doubtful record on sugar cane in England (*8*) and the record of its occurrence in the oogonial stage in tassels of maize in South America (*16*)—a case in which it seems more probable that the fungus was *Sclerospora macrospora*. The variety *S. graminicola* var. *andropogonis-sorghi* of Kulkarni (*7*) on sorghum, as has been pointed out also in a previous paper (*21*), is sufficiently distinct to warrant its being established as a separate species.

In the United States, *Sclerospora graminicola*, since it was reported first by Farlow in 1884 (*4*) on the green foxtail grass, *Chaetochloa viridis*, from La Crosse, Wis., has been recorded chiefly on that host, although there have been a few records of its occurrence on the closely related smooth foxtail grass, *C. glauca* (L.) Scribn., and several reports of it on the cultivated Italian millet, *C. italica* (L.) Scribn., and on the German or Hungarian variety, *C. italica* var. *germanica* (Mill.) Scribn. These records are chiefly from the northernmost line of States, New York, Michigan, Wisconsin, Minnesota, and North Dakota, with some records also from Iowa, Illinois, and South Dakota, while, in so far as the writers have been able to determine, the most southern records hitherto have been from Kansas and New Mexico.

To these previous records this new note of the occurrence of *Sclerospora graminicola* on *Chaetochloa magna* in Florida is an addition of considerable significance. Hitherto the fungus has been found in the United States, not on native hosts but on hosts which have been introduced from Europe, even the widespread and well-established *C. viridis* being of European origin. This has suggested strongly that the parasite, as it was found only on these hosts, was of European origin as well. The widespread distribution in Florida, however, on such an indigenous grass under wild conditions seems to justify the assumption that the fungus also may be native to the

southeastern part of the United States. The other possibility, that at some time in the past the fungus may have escaped from cultivated millet and become established on this wild host, seems opposed by the fact that millet has been and is very little cultivated in the State, and that the fungus on the wild host under natural conditions is so generally distributed and apparently so long established in wild localities remote from cultivation.

Indeed, so regularly is the fungus associated with *Chaetochloa magna* in Florida that it seems highly probable it will be found on this host elsewhere. The geographic range of the grass in the United States is not limited to Florida, but extends along the southern Atlantic seaboard from Delaware to Florida and along the Gulf States from Louisiana to western Texas, while outside of the United States the grass is known in Bermuda, the West Indies, and Central America. From the fact that the occurrence of this downy mildew parasite in Florida is so precisely coincident with the occurrence of the grass host, the writers feel that it is justifiable perhaps to expect that this same coincident occurrence will be found in other parts of the range of the grass.

SYMPTOMS AND EFFECT

The symptoms which Everglade millet displays when infected by *Sclerospora graminicola* are relatively easily recognized. Infection most commonly is systemic, and the symptoms agree in general with those characterizing the attack of this fungus on other hosts. Also, however, local infection restricted to scattered spots on the leaves was found to occur—a less obvious type of infection which, as far as the writers are aware, hitherto has not been recorded for this fungus.

Systemic Infections

Systemic infection shows itself chiefly in the development of pallid yellowish markings in the dark-green tissue of the leaf. (Pl. 1, A, D, and E.) On the lowest (i. e., oldest, earliest unfolded) leaf thus marked, the pallid area is restricted to the base of the leaf; but on each successive leaf unfolding thereafter the areas are more extensive, running out in irregular, jagged extensions, progressively farther and farther toward the tip, the latest leaves being completely yellowish white throughout. The characteristic markings on successive leaves of a typically infected plant are shown in the accompanying diagram (fig. 2), which was prepared by tracing carefully with a sharp stylus through the tissue of spread-out leaves. These markings may appear very early in the life of the plant or when it is half grown or older, the first (lowest) leaf to show these areas being any leaf from the third or fourth to the eighth or ninth. Seedlings that are severely attacked are readily distinguished from their healthy fellows on casual examination, even when only a few inches tall, by their pallid, marked appearance. (Pl. 1, A.) Young plants that are growing rapidly may be a foot or two tall before the fungus invades the later unfolding leaves and they become symptomatically pallid.

In general these symptomatic markings of the affected plants resemble those characteristically produced by other members of the

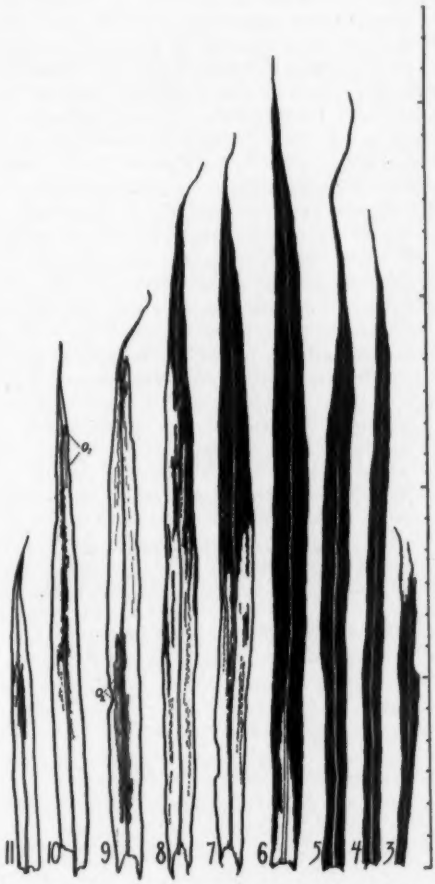


FIG. 2.—Characteristic markings of systemic infection. Diagrams showing the typical configuration and extent of the etiolated conidiophore-bearing areas on successive leaves of a *Chaetochloa magna* plant systemically infected by *Sclerospora graminicola*. Drawn from life-sized tracings, scale in inches. The formation of resting spores (oogonia containing oospores) already has begun in the tissue of the older leaves in the stippled spots at O_1 and O_2. The affected, etiolated area (white) is increasingly greater and the unaffected area (black) increasingly smaller on successive leaves. The actual and the proportionate extent of these areas was as follows:

Item		Leaf No.										Total
		11	10	9	8	7	6	5	4	3	2 and 1 withered	
Leaf area	sq. cm.	35.2	69.78	89.76	107.60	123.86	134.9	87.38	61.80	23.1	0	733.38
Conidiophore-bearing area	do	35.2	69.78	89.76	62.88	46.86	13.5	0	0	0	0	317.98
	per cent	100	100	100	58.44	37.83	10	0	0	0	0	43.36

genus Sclerospora that have been studied intensively. The similarity between the pattern and configuration of these areas, shown here in Figure 2, and those figured in earlier papers (*19, pl. B; 20, fig. 1*) for the Philippine mildews of maize, is quite striking. The actual area of these symptomatic markings, being proportionate to that of the large coarse leaves of the Everglade millet, is quite extensive, even as large as 317.98 sq. cm. in the case of some medium-sized systemically infected plants. (See Table 1.) As the production of conidiophores from the mycelium within the host takes place on these areas, the extent of this production also can be gauged and compared. In such a comparison (Table 1) the conidiophore-bearing area of leaves of Everglade millet systemically infected with *Sclerospora graminicola*, although this production takes place on only one surface, is found to compare very well with similar areas on maize plants systemically infected with *S. philippinensis*, even though in maize the production of conidia customarily is on both sides of the leaf. In this comparison the areas of the leaf sheaths as formerly recorded (*20, Table 2*) are omitted to make the comparison more just, for little if any production of spores takes place on the leaf sheaths in Everglade millet.

TABLE 1.—*Conidiophore-bearing areas on representative host plants infected by species of Sclerospora*

Comparison of the extent of sporulating areas on (1) *Chaetochloa magna* plants, showing inconspicuous local-spotting type of infection with *Sclerospora graminicola;* (2) *C. magna* plants showing the conspicuous systemic type of infection with *S. graminicola;* and (3) *Zea mays* plants of representative sizes of two varieties showing systemic infection with *S. philippinensis* previously recorded by Weston (*20, Table 2*)]

Items compared, variety, and plant number	Number of leaves	Approximate height of plant (cm.)	Total area of leaves (sq. cm.)	Conidiophore-bearing area	
				Sq. cm.	Per cent
Local infections on C. magna by S. graminicola:					
No. 1	8+2 withered		[a] 925.60	[a] 3,665	3.96
Systemic infections on C. magna by S. graminicola:					
No. 2	9+2 withered		[a] 733.38	[a] 31,798	43.36
Systemic infections on Zea mays by S. philippinensis:					
Native Yellow Flint, No. 5	9	91	[b] 1,819.02	[b] 35,170	19.33
Native Yellow Flint, No. 10	12+ tassel	192	[b] 5,127.90	[b] 262,920	51.27
Mexican June, No. 11	9+ tassel	126	[b] 1,502.78	[b] 134,655	89.60
Mexican June, No. 16	do	154	[b] 3,656.79	[b] 128,580	35.16

[a] Lower surfaces only. [b] Both surfaces.

On these pallid areas there develops during favorable dewy nights a downy growth of conidiophores and conidia (zoosporangia), the presence of which, of course, is an additional and conclusive symptom.

When plants show the disease while still small seedlings, presumably as a result of very early infection, they may be killed in a relatively few weeks or may struggle on for some time without developing any additional destructive symptoms.

When plants, presumably after much later infection, do not show the disease until partly grown, the effects of the parasite run on in a further symptomatic course. Such plants as they mature usually become distinctly stunted in comparison with the healthy ones (pl. 2,

A), and in some cases also put out an abnormal abundance of suckers that give a bunchy, rosetted appearance to the clumps (pl. 2, B). As a rule, if one shoot from a clump shows systemic infection, all those developing subsequently will do likewise. (Pl. 1, B, C.)

On the Everglade millet, as on its other hosts, the fungus runs through the two typical reproductive phases of its life history. On the pallid areas occurs first the formation of the conidial stage, which continues on the leaves, already unfolded, during favorable dewy nights and begins anew on the pale portions of the leaves that develop subsequently. In time, however, as the host matures, the production of conidiophores on the surface of the leaves lessens and gradually gives way to the formation of oogonia from the mycelium within the host tissue. The production of oogonia usually begins in the older, lower leaves and goes on progressively upward in leaf after leaf until established last in the topmost, youngest leaves, which may continue to support conidiophore production even until the terminal inflorescence is developed and long after the lower leaves are riddled with oogonia.

Of this oogonial phase of the disease certain additional symptoms are characteristic. When the intramatrical hyphae begin to form oogonia, the infected areas of the host tissue in which they are located change from the pallid, yellowish color that has characterized them to a somewhat reddish brown. In time the leaf tissue between the bundles disintegrates (pl. 2, E, F), the bundles persisting as the leaves become shredded, until only a tangle of frayed fibers is left (pl. 2, C). Usually, also, the inflorescence or head of the grass is not normally developed, but is decidedly stunted and virescent or otherwise teratologically malformed (pl. 2, D), much as are those so carefully described by Butler (*2*) in the case of *Pennisetum typhoideum* when attacked by this fungus in India. As a rule such heads are sterile, and the florets, instead of producing seed, grow into small clusters of bracts, the palea and lemma particularly tending to develop abnormally into straplike or leaflike growths. These abnormal structures are permeated also by the mycelium of the parasite, and they may for a time support production of conidiophores. Ultimately they fray out into frazzled fibrous tangles as the interfascicular tissue disintegrates with the maturing of the oogonia.

Local Infections

In addition to the usual systemic type of infection just considered, there occurs on the Everglade millet, as has been mentioned above, a very different, local type of infection. In this type the infection of the host is limited to restricted spots on the leaves. While the cases of systemic infection immediately attract attention because of the conspicuous symptoms of the disease—the obvious, pallid markings, stunted growth, and malformed inflorescences—the locally infected plants show no striking symptoms and in consequence are likely to escape notice. They are relatively numerous, however, many fields of the grass showing hardly a single apparently healthy plant that on close examination does not have at least a few scattered spots of local Sclerospora infection on its leaves. In one

typical field near Miami, along Cocoplum Beach Road in low land formerly under cultivation but grown up to a rank growth of Everglade millet when examined, 202 plants chosen at random were inspected carefully. Only 2 were found to be quite healthy without any signs of Sclerospora infection whatever, while 149 showed more or less abundant spotting of local infection, and 51 displayed obvious symptoms of systemic attack.

Although no extensive counts were made, some attention was paid to the relative frequency of occurrence of both types of infection around Miami, and the proportion given above was found to be a fairly typical one. A very large proportion of the tall vigorous plants that on casual examination appeared to be entirely free from Sclerospora were found on closer inspection to have at least a few local infection spots, chiefly on the upper, younger leaves. In some cases both types of infection were found on the same plant, the plants systemically attacked showing also scattered, locally infected spots either near the base of the plant on the wholly green lower leaves below those that showed the progressively pallid markings typical of systemic infection, or even on these leaves themselves, although only on their green unaffected tips. In no cases were local infection spots found in the pallid areas of leaves already invaded by the mycelium of systemic infection.

From the healthy, normal green, uninvaded tissue of the leaves the invaded locally infected spots were distinctly defined by being somewhat sunken, of a rather irregularly roughened surface texture, and of a buff to dull reddish to very dark-brown color.

In shape and size these spots of local infection varied from irregular, somewhat rectangular, areas of only a few square millimeters in extent to extensive linear streaks running lengthwise of the leaf. (See fig. 3.) In general, rather sharply bounded laterally by the leaf veins (though sometimes irregularly jagged), and varying from 1 to as much as 10 mm. in width, they are relatively elongate, ranging from one-fifth to perhaps even 10 cm. in length.

EXPLANATORY LEGEND FOR PLATE 1

A.—Young plant of Everglade millet showing systemic infection with *Sclerospora graminicola*. Beginning with the fifth leaf, the characteristic elongate, pallid markings are clearly evident, and on them the production of the down of conidiophores is begun. About one-fourth natural size. (Photographed at Coconut Grove, Fla., June 22, 1924, by G. F. Weber.)

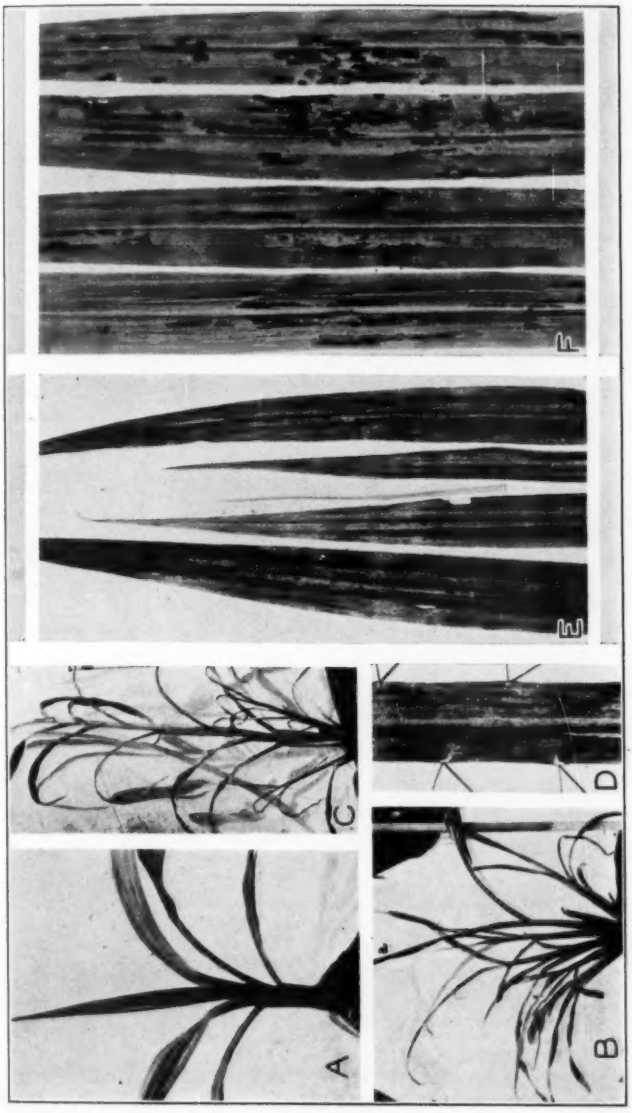
B.—A young Everglade millet plant of six shoots in which infection by the downy mildew presumably occurred after the last largest shoot (*a*) already was developed, as this one is healthy while the other five younger, smaller, more recently developed shoots all show peculiar systemic general infection by the downy mildew. (Photographed along the Cocoplum Beach Road in June, 1924, by W. H. Weston, jr.)

C.—A vigorous yet badly infected Everglade millet plant, the largest 3-foot shoot of which shows characteristic systemic infection of the downy mildew and is still supporting conidiophore production on its leaves, while in the two smaller and stunted systemic-infected shoots at its base the production of conidiophores has ceased and already the formation of the resting spores in the oogonia has begun in the curled and drying leaf tissue. (Photographed along the Cocoplum Beach Road in June, 1924, by W. H. Weston, jr.)

D.—Portion of the upper surface of the eighth leaf of a systemically infected plant with fairly conspicuous symptomatic etiolated conidiophore-bearing areas. × ½. (Photographed at Coconut Grove, Fla., June, 1924, by W. H. Weston, jr.)

E.—Successive leaves of Everglade millet showing the elongate pallid markings of systemic infection by the downy mildew with conidiophore production still taking place on them. (Photographed at Florahome, Fla., April, 1924, by G. F. Weber.)

F.—Successive leaves of large Everglade millet plant showing the characteristic irregular spotting of the local type of infection with the downy mildew. (Photographed at Florahome, Fla., April, 1924, by G. F. Weber.)



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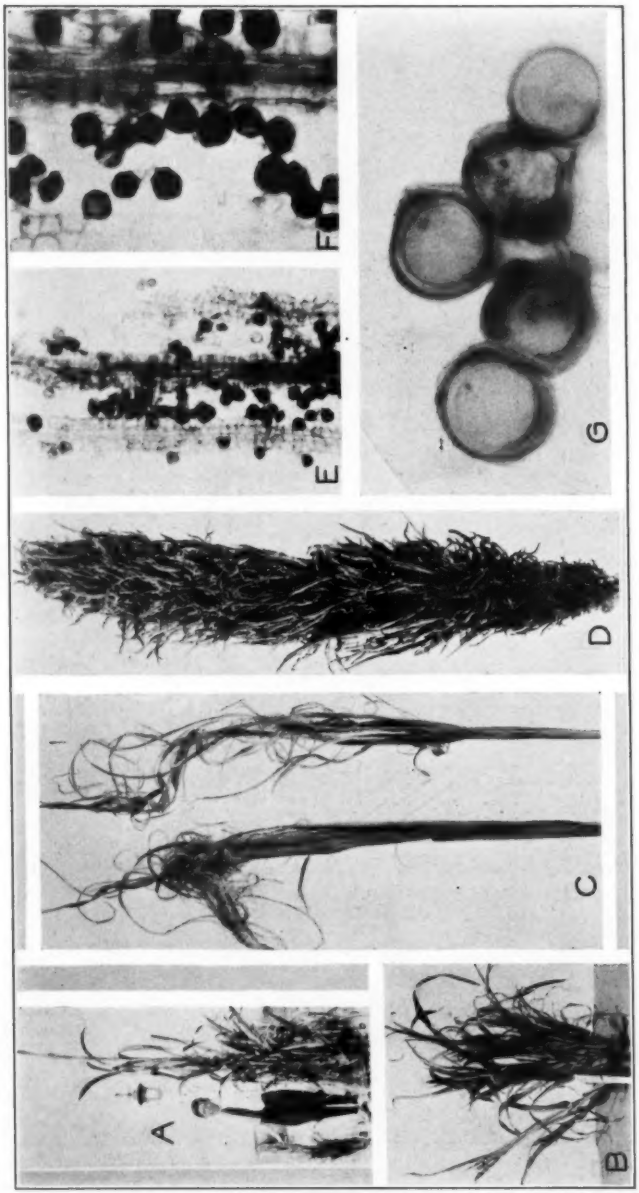

(For explanatory legend, see page 943)

The spots occurred not only separately and scattered but also coalesced into larger areas of more or less irregular outline. Their distribution on the leaves showed no definite pattern or arrangement but was decidedly irregular.

The characteristic appearance of these areas of local infection is well brought out in Plate 1, F. Their size, shape, and arrangement also are shown in that illustration and in the outline diagram of Figure 3, which was traced from a typically representative, locally infected plant. The actual area of these locally infected spots is relatively small, occupying in a typical case such as the one tabulated, only 36.65 sq. cm., or a little less than 4 per cent of the total leaf area. Obviously (see Table 1), this is relatively small, also, compared to the much greater extent of the area that is etiolated and produces conidia in the case of the systemic infections.

The production of Sclerospora conidiophores took place almost exclusively from the undersurfaces of these spots, and under favorable circumstances the fungous growth could be seen as a sparse down recognizable with a hand lens. The conidiophore production was always rather scanty in density and abundance compared to that on the etiolated tissue of systemically infected plants. As the plants aged, the locally infected tissue merely ceased to produce conidia and, so far as was observed, dried up without ever developing oogonia or fraying out with the disintegration of interfascicular tissue that accompanies this process.

On the whole, there was nothing outstandingly distinctive about the symptomatic markings of this local type of infection, the spots to the casual glance resembling those caused byHelminthosporium, for example. The facts that it is not outstandingly distinctive or conspicuous and that it at first escaped their attention in fields where it was very common lead the writers to suspect that, although

EXPLANATORY LEGEND FOR PLATE 2

A.—A comparison of a normal-sized, vigorous, fruiting, healthy clump of Everglade millet (*b*) with the characteristically stunted, poorly developed, sterile plant heavily systemically infected with *Sclerospora graminicola* (*a*). The boy holding the plant is about 4½ feet tall. (Photographed at Coconut Grove, Fla., June, 1924, by W. H. Weston, jr.)

B.—A stunted plant of Everglade millet with the dense bunchy growth which results from early and long-standing infection with downy mildew, now given over almost wholly to the production of oogonia in its tissues. (Photographed at Coconut Grove, Fla., June, 1924, by W. H. Weston, jr.)

C.—Characteristically shredded fibrous tops of plants which have been killed by the downy mildew and now in the late stages of the disease are given over to the production of oogonia, with a disintegration of the tissue between the bundles and the consequent fibrous tangles of the dry bundles from which the resting spores are constantly scattering. About two-thirds natural size. (Photographed at Belle Glade, Fla., July, 1924, by G. F. Weber.)

D.—The head or inflorescence of the Everglade millet plant badly infected by the downy mildew, showing the abnormal development of elongate, strapilike bracts in the place of the normal parts of the flowers, so that the whole head is sterile and virescently malformed as a result. The tissue is full of oogonia but has not as yet shredded out to set the spores free. (Photographed at Pomona, Fla., May 28, 1924, by G. F. Weber.)

E.—Tissue of a portion of the leaf of Everglade millet showing the location of the oogonia in the disintegrating tissue between the fibrovascular bundles. × 75. (Photomicrograph by S. A. Howes.)

F.—A more highly magnified view of the same sort of leaf tissue which has begun to shred out in the late stages of the disease, showing the position of the resting spores in their relation to the bundles. × 125. (Photomicrograph by S. A. Howes.)

G.—Resting spores (oospores) surrounded by the closely enveloping, thickened, irregular, oogonial wall; cleared and photographed in lacto-phenol solution to show the structure of the oospores and their relation to the enveloping oogonia. × 350. (Photomicrograph by S. A. Howes.)

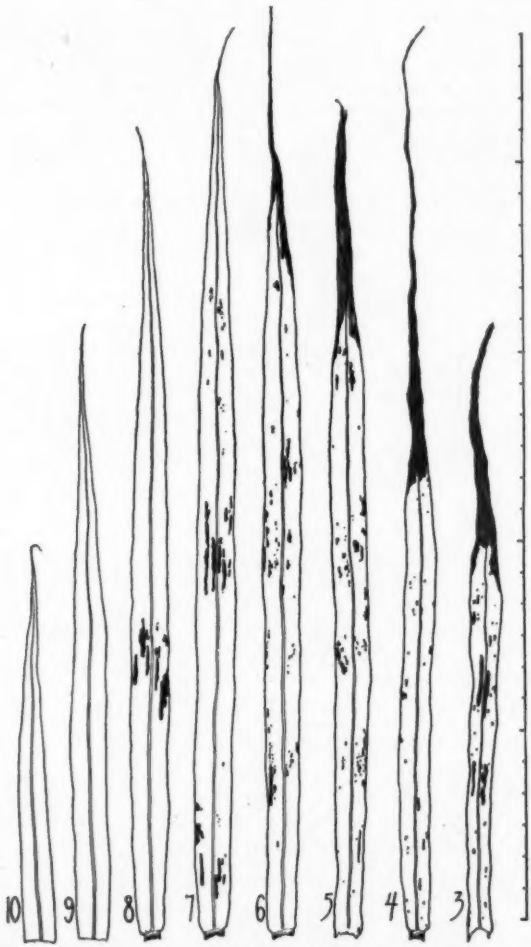

FIG. 3.—Characteristic markings of local spot infection. Diagrams showing the typical shape, size, and distribution of the reddish-brown discolored areas bearing conidiophores on successive leaves of a *Chaetochloa magna* plant locally infected by *Sclerospora graminicola*. As is customary with this type of infection, no formation of resting oospores occurred. Drawn from life-sized tracings, scale in inches, the spots distinguished by shading from the unaffected leaf tissue (white). The irregularity in the distribution of the spots and in the area they occupy on different leaves is distinctly in contrast to the situation in systemic infection. The actual and the relative extent of these areas was as follows:

Item		Leaf No.									Total
		10	9	8	7	6	5	4	3	2 and 1 withered	
Leaf area	sq. cm.	49.96	93.22	133.4	167.4	155.06	141.08	105.78	79.7	0	925.60
Conidiophore-bearing area	do	0	0	4.97	9.19	6.82	7.09	2.48	6.1	0	36.65
	per cent	0	0	3.7	5.5	4.4	5.0	2.3	7.6	0	3.96

apparently not yet reported, this local infection by *Sclerospora graminicola* perhaps occurs on other hosts and merely has remained unnoticed. In any case, its occurrence on Everglade millet is of considerable interest, first, because of its possible significance, and second, because of its possible importance in the distribution of the disease. That *Sclerospora graminicola* should so frequently manifest itself in this local type of infection, so different from the customary systemic one, suggests either that a different strain or physiologic race is responsible or that the same strain which usually accomplishes systemic infection may produce very dissimilar effects on the host under different conditions. If, indeed, a distinct strain is concerned, one would expect to find the physiological difference in the activity of the strain accompanied by recognizable though perhaps slight distinctions in such morphological criteria as the size of the conidia. While a slight difference was indeed found in size of the conidia from local infections in comparison to that shown in Table 2, it was not regarded by the writers as sufficiently conclusive evidence of a distinct strain; (1) because it was very slight (only 2 μ longer and 2 μ broader); (2) because it was based on so few measurements (only 50 spores, from one plant, during one night in the scanty opportunity for night work at Miami in June, 1924); and (3) because it was not accompanied by any difference in shape (the most common ratio of length over width remaining the same, 1.25 to 1.34) or by any difference in the essential size or structure of the conidiophores. Further measurements should be made to settle this point. With the evidence at hand, however, it seems much more probable to the writers that the same strain of *Sclerospora graminicola*, acting under different conditions, produced both local and systemic infection.

The impression gained by extensive field observations is that the point of infection and the age of the host are instrumental in determining the type of infection that will result. Apparently, if inoculation takes place by spores falling into the unfolding, dew-containing terminal bud, the resulting attack will be systematic, even if the plant is relatively well grown. Also, if the spores fall in or very near the leaf axils of very young seedlings, the infection may reach the growing center and become systemic. If, however, the spores fall upon the fully differentiated, mature, and more resistant tissue of well-grown mature leaves of older plants, they will give rise to local mycelia which have only a limited restricted growth as locally infected spots. These reactions of the fungus, inferred from field observations, were not verified by inoculation experiments; but it should be noted that in the case of the conidial Sclerosporas of the Philippines the development under differing conditions of infection has been found, in work not as yet published, to be that described above.

The other interesting aspect of the local type of infection is its possible importance in the distribution of the disease. Although relatively inconspicuous and supporting relatively scanty spore production in comparison with the systemic type, the local infection, because of its common occurrence and its persistence throughout the

year, plays an active part in spreading the Sclerospora, a point that should be borne in mind in connection with the question of its economic importance and control.

THE CAUSAL ORGANISM

ITS IDENTITY

As was to have been expected from its restriction to gramineous hosts and the nature of its effect on these hosts, the causal organism proved to be a member of the peronosporaceous genus Sclerospora, a genus noted for its destructive parasitism of the Gramineae. As the fungus occurred in luxuriance, it was readily possible to follow the development of both the conidial (zoosporangial) and the oogonial stages, to collect an abundance of material of both phases, and to make the numerous measurements of sizes of spores and other structures necessary to serve as an adequate basis for identification. On this basis the fungus was determined to be *Sclerospora graminicola* (Sacc.) Schroet., a species of wide distribution, but one not reported hitherto from such Southern States as Florida or on this species of grass.

STRUCTURE, DEVELOPMENT, AND RELATION TO ENVIRONMENTAL CONDITIONS

The mycelium of the fungus in cases of systemic infection is very generally distributed through the tissue of the host, occurring especially abundantly in the pallid areas of the symptomatically marked leaves (see pl. 1, A, D, E) and to a lesser degree in the leaf sheaths, in the inflorescences, and in the stem both above and below the ground, but not in the roots. In cases of local infection the mycelium is restricted to the tissue of the discolored spots. (See pl. 1, F.) In either case the mycelium is intercellular in position, comprises slender hyphae of transmission, running from place to place along the bundles, and knotted, much-branched, contorted, feeding hyphae which are crowded into the interstices between cells of the mesophyll and furnished with short knob-shaped or finger-shaped haustoria.

From this mycelium are developed the two successive reproductive phases of the fungus, the treelike conidiophores that grow out externally from the stomata, and the large thick-walled oogonia or resting spores that are formed later within the disintegrating host tissue.

THE CONIDIAL STAGE

Conidiophore production on this host and under subtropical conditions in Florida, like that already described for this fungus on foxtail grass in Minnesota (*21*), takes place at night when the surfaces of the mycelium-invaded tissue are covered with dew or other moisture, a relationship that has been considered in more detail in earlier papers (*19*, *20*, *21*). Preliminary to conidiophore production the mycelium among the mesophyll cells gives rise to thick, irregular clusters of hyphal branches closely crowded in the air chambers beneath the stomata from which the conidiophores will arise. The subsequent development of the conidiophores and conidia agrees with

the process as it has been described already for this Sclerospora on *Chaetochloa* (*Setaria*) *viridis* in Minnesota. This agreement holds in all phases of the process from its beginning with the emergence from the stomatal slit of branches which form a compact group of bulbous knobs, elongate into club-shaped stalks, and develop by further elongation and branching into the conidiophores, up to and through the final production of conidia (zoosporangia) on the terminal sterigmata of the mature conidiophore and the subsequent collapse of these when the conidia have been shed.

The Conidiophores

The descriptions and drawings of all these stages of conidiophore production, as they were found to occur on *Chaetochloa* (*Setaria*) *viridis* in Minnesota (*21*), would serve with equal accuracy to depict the process as followed on *C. magna* in Florida. Also the distinctive mature coniodiophores (fig. 4, A, B, C, D) agree in such critical morphological features as the size and shape of the main axis, with its slightly differentiated basal foot (fig. 4, E to J); the structure, number, and position of the branches; the shape and arrangement of the sterigmata, and the structure of the spores borne on them (fig. 4, K to O). This agreement is well shown if the accompanying drawings of typical, well-developed conidiophores under optimum nocturnal conditions (fig. 4) are compared with similar conidiophores illustrated for *Sclerospora graminicola* in Minnesota (*21*, *pl. 1*, *2*). As in the material of *S. graminicola* studied in Minnesota, the basal cell, which is such a consistently developed feature in the Philippine Sclerosporas, is the exception here. (See fig. 4, E to J.)

The Conidia

The conidia, in all the essential distinguishing features of size, shape, structure, and germination, show the characteristic peculiarities that mark *Sclerospora graminicola* as such a distinct species.

In size the conidia, like those of the downy mildews in general, show considerable individual variation. The size should be presented quantitatively, therefore, from large numbers of measurements, if it is to be adequate for identification or comparison. Taking advantage of the facilities for night work that were generously offered by Hugh Matheson at Coconut Grove, one of the writers measured 250 conidia on the nights of June 16 and 17, 1924, under optimum conditions, during maximum production from 1.30 to 3 a. m. They were measured while fresh and living in the dew in which they had been shed. In making previous diagnostic measurements of this species in Minnesota in 1920, it had been necessary (*21*) to use preserved material. This, although killed very carefully in Flemming's weaker solution and mounted in dilute glycerin, shrank slightly, so the conclusion was reached that "a comparison of measurements made thus with the relatively few which the writer had opportunity to make from fresh material indicates that if all the measurements had been made under ideal conditions the modes of length and of diameter probably would have been increased by one

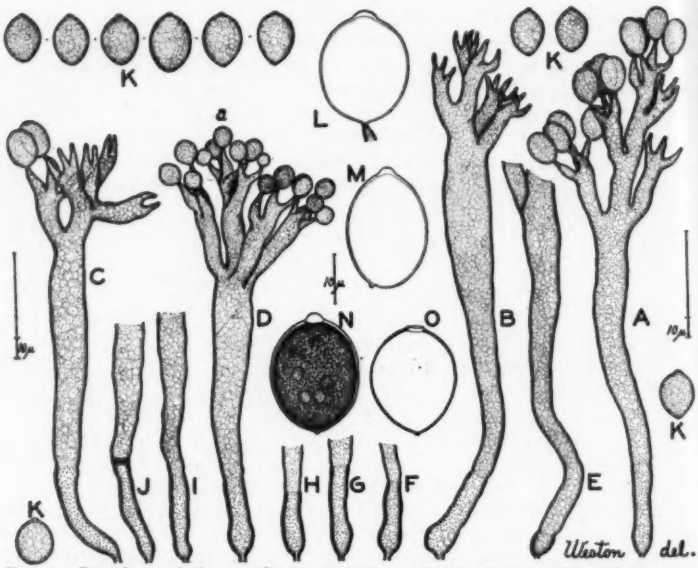

FIG. 4.—Drawings of the conidiophores and conidia (zoosporangia) of *Sclerospora graminicola* on *Chaetochloa magna*. Prepared with the aid of a camera lucida and with the exception of K (made from living spores just shed in dew) made from material scraped from leaves at the time of maximum noctural production of conidiophores, immediately killed with Flemming's weaker solution and stained with eosin, or killed and stained at the same time with picronigrosin; then mounted in dilute glycerin. The approximate magnification of the printed figures after reduction from the original drawings is given in each case; also a 10 μ scale is shown as an absolute measure.

A.—A conidiophore in which is especially accentuated that continuation of the main axis through the branch system which characterizes the species. The zoosporangia are only partly grown, as is obvious from their size and structure; from the two branches with truncate tipped sterigmata at the right they have been broken off. ×375

B.—A partly grown conidiophore in which the branch system is already complete and the ultimate tips have elongated into the tapering sterigmata from which the zoosporangia will bud out. As is customary in the species, the base is differentiated by thickening of the wall into a footlike portion. ×375

C.—A rather scanty conidiophore which has lost all but two of its zoosporangia and shows clearly the usual structure of the branch system with its continuing main axis. ×375

D.—A well-developed though rather stocky conidiophore which in contrast to those shown in B and C has its several branches of approximately equal size and rank coming off almost at the same point from the main axis (which can, however, be seen continuing through to *a*). This type of branching is occasionally encountered and represents for the species one extreme of the range of forms of which the type of A, with its pronouncedly excurrent main axis, is the other extreme. ×375

E.—Main axis of a conidiophore showing at its base an unusually short footlike differentiation. ×375

F–I.—Bases of five conidiophores showing differentiated footlike portions which in extent and thickening of the wall are typical of the species. ×375

J.—Base of a conidiophore with a complete basal cell cut off by a cross wall, a rare condition in *Sclerospora graminicola*, though typical in the conidial oriental species. ×375

K.—Ten representative conidia (zoosporangia) showing the range of shapes and sizes most frequently encountered. Note that each has the terminal papilla of dehiscence essential to its germination by the emission of zoospores. ×375

L.—A mature zoosporangium just before being shed from its sterigma, drawn in outline to show the structural modification of the wall at the tip into the dehiscence papilla and at the base into the apiculus of attachment to the sterigma. ×375

M.—Outline drawing of a mature zoosporangium recently shed from its sterigma and showing the basal apiculus of attachment located, as it occasionally is, to one side of the median line. ×850

N.—A mature, recently shed zoosporangium with its apical papilla of dehiscence well developed, its basal apiculus of attachment still discernible, and its granular content, stained with picronigrosin, still relatively undifferentiated before division into zoospores. ×850

O.—Empty wall left by such a zoosporangium as N after it has germinated by emitting zoospores through the pore left by the softening of the terminal dehiscence papilla. ×850

2 μ class (from 18 to 20 μ and from 14 to 16 μ)" (*21*). It is of interest that the measurements in Florida of living, freshly shed spores closely bear out this prediction, as the modes of length and of diameter fall in the 22 μ and the 16 μ classes, respectively. Florida conidia secured at the same time, but mounted in dilute glycerin, shrank slightly and gave the same smaller measurements as the preserved Minnesota material. As might be expected, since the shrinkage was approximately even, the ratios of length over diameter were not altered, the mode falling into the ratio class of from 1.25 to 1.34 in both preserved and living spores.

It is of interest that the diagnostic conidial measurements of this species on *Chaetochloa* (*Setaria*) *viridis* in St. Paul, Minn., in July, 1920, and on *C.* (*Setaria*) *magna* in Coconut Grove, Fla., in June, 1924, agree so closely. This agreement seems to indicate that such a quantitative expression of spore size is indeed a dependable distinguishing character of the species and not appreciably altered by development under very different climatic conditions or on different hosts. The table of spore measurements of living material here presented (Table 2) may be regarded, then, as accurately representing the conidia of *Sclerospora graminicola.*

TABLE 2.—*Measurements and ratios of length to diameter of conidia (zoosporangia) of Sclerospora graminicola arranged in size and ratio classes, comparing those of living material from Chaetochloa magna in Florida in 1924 with those of preserved material from C. viridis in Minnesota in 1920 previously recorded by Weston (21)*

Classes	Florida 1924 living material, number of conidia in 250	Minnesota 1920 preserved material, number of conidia in 400	Classes	Florida 1924 living material, number of conidia in 250	Minnesota 1920 preserved material, number of conidia in 400
Length:			Diameter—Continued		
11 to 12.9 μ	0	7	13 to 14.9 μ	39	162
13 to 14.9 μ	2	25	15 to 16.9 μ	105	92
15 to 16.9 μ	11	67	17 to 18.9 μ	72	30
17 to 18.9 μ	35	119	19 to 20.9 μ	22	4
19 to 20.9 μ	59	89	21 to 22.9 μ	7	0
21 to 22.9 μ	60	51	23 to 24.9 μ	1	0
23 to 24.9 μ	46	22	Ratio, length to diameter:		
25 to 26.9 μ	17	10	0.95 to 1.04	0	1
27 to 28.9 μ	6	6	1.05 to 1.14	11	31
29 to 30.9 μ	4	1	1.15 to 1.24	87	75
31 to 32.9 μ	0	1	1.25 to 1.34	119	115
33 to 34.9 μ	0	2	1.35 to 1.44	26	92
35 to 36.9 μ	1	0	1.45 to 1.54	4	46
Diameter:			1.55 to 1.64	3	25
9 to 10.9 μ	0	1	1.65 to 1.74	0	9
11 to 12.9 μ	4	111	1.75 to 1.84	0	6

It also is of interest that the few measurements of conidia from locally infected plants show a preponderance of larger sizes compared to the representative measurements just considered from systemic material. This material was comparable from the point of view of source, as it was taken from locally infected plants of approximately the same age, subjected to similar conditions as regards location, temperature, and moisture. It might, therefore, be

regarded as significant, an indication of a distinct strain of *Sclerospora graminicola*, only slightly different morphologically from the type, but decidedly different physiologically in its effect on the host. It seems doubtful to the writers, however, that this interpretation is the correct one. In the first place, as has been discussed earlier, the symptoms as studied in the fields seem much more probably to be the result of later infection of more mature parts of the plants. In the second place, the 50 measurements of conidia from local spots are too few to give an adequate basis for comparison, for if the 250 measurements of conidia from typical systemically infected plants are examined in lots of 50, some of these lots show as great a departure from the size distinctions established by the total as do the measurements of the local spot material. Moreover, as was noted above, the difference is one of slight increase equally in all dimensions and not accompanied by any difference in shape, the ratio of length divided by diameter being the same.

In shape the conidia are most frequently broadly ellipsoidal or rotund cylindrical, but they show considerable variation. A qualitative idea of the usual range is given by the illustrations of the representative shapes of 10 spores in Figure 4, K, while a quantitative conception of the frequency of the broadly ellipsoidal form (with a length from 1.25 to 1.34 times the diameter) is brought out clearly in the tabular presentation of the ratios of length over diameter. (Table 2.)

The structure of the conidia (fig. 4, L to O) agrees with that already described for the species (*21*) in the details of the thin cellulose wall, of the specialized apical papilla of dehiscence, and the granular content with its several nuclei.

On germination the few to several zoospores into which the content has developed escape through the terminal pore left by the gelatinizing of the apical papilla. This method, essentially zoosporangial, is the typical one by which the spores liberated during the night into dew or other moisture germinated under natural field conditions. It took place rapidly and universally at temperatures from 70° to 80° F. on leaves or other plant parts, or on slides in drops of dew, rain, or tap water. At 50° in an ice box it was the only method obtaining in the very low percentage of slow germination that did occur. At 90° or 92° no germination took place and the content of the spores became somewhat clotted and vacuolate and seemed irreparably injured. Very rarely, in exceedingly few cases of the large number observed, the spores germinated by putting out a short abortive hypha, presumably in response to conditions unfavorable for normal germination, as the few instances occurred in the laboratory in the mounts that became unduly warm (85° plus) when observed over a substage lamp and in material collected in nature in scanty moisture on leaves drying in a warm breeze at dawn.

THE OOGONIAL STAGE

In Florida, on the Everglade millet, as elsewhere in the world on other hosts, the life cycle of this downy mildew involves an oogonial as well as a conidial stage, both stages usually occurring in the life cycle with neither one developing to the exclusion of the other.

The occurrence of the oogonial stage usually follows the conidial, hence the oogonia customarily develop on the lower, older leaves after the formation of conidia thereon has ceased, even though the latter still may be developing on the upper, more recently expanded leaves of the same plants. Occasionally, however, the oogonia may develop in profusion together with the conidia on the same leaf; and usually for a time during the transitional period when any leaf is ceasing to develop conidia and beginning to form oogonia, some of each may be found upon and in it.

The oogonia are developed within the tissue of the host, chiefly in the leaves, but to a less degree in the leaf sheaths, or in the modified leaflike structures which form in malformed badly infected heads. (Pl. 2, D.) On these parts of the plant the oogonia are formed by the mycelium chiefly where it is most abundantly developed between the disjoined cells of the spongy mesophyll and of the bundle sheaths around the fibrovascular bundles. (Pl. 2, E, F.) As the formation of the oogonia proceeds, the host cells in these regions are forced apart, crushed, and destroyed progressively until ultimately through this disorganization and withering of their tissue the leaves become shredded by longitudinal splitting between the bundles, leaving these as delicate, threadlike fibers, at first more or less parallel in a brushlike arrangement, but later tangled and snarled into knotted masses. (Pl. 2, C.) From the disintegrating interfascicular tissue the oogonia, which by now are mature, shake out and are scattered very effectively by even the slightest quivering of the plant in the wind. So characteristic is the shredding of the leaves in the latter stages of the disease that it is one of the most distinctive symptoms of this downy-mildew infection of Everglade millet in Florida, as it is on other hosts in other parts of the world.

The formation of the oogonia by the mycelium within the tissue agrees in its general features with the early description of Schroeter (*13*) in the case of *Setaria viridis*, and of Butler (*2*) in *Pennisetum typhoideum*. By cutting sections or macerating the tissue, the swelling of the terminal or intercalary oogonia and the attachment of antheridia from near-by branches of the mycelium were easily followed, the process in gross details and in its minute cytological features agreeing closely with the careful account given by Stevens (*17*) for this same species in *S. viridis*.

The oogonia when fully mature are quite distinctive. In color they range from a somewhat pallid amber through deeper reddish tones approximating the color of dark rosin, or, according to Ridgway's (*11*) color scheme, from pale ochraceous orange, through zinc orange and xanthine orange, to orange rufous and amber brown. Perhaps the most common coloration is that of rather dark reddish rosin, which agrees rather closely with the shade called orange rufous by Ridgway. The single oospore within the oogonium, on the contrary, has a somewhat pallid, almost hyaline wall, sometimes slightly tinged with a pallid golden or amber tone. The content has chiefly the pallid, somewhat gray shade of protoplasm, but may contain here and there oil globules that are generally slightly golden.

In shape the oogonia vary greatly, being in general somewhat rounded polygonal, their outlines conforming largely to the shape of

the intercellular spaces in which they have formed. (Pl. 2, F.) The oospore itself, however, is approximately spherical with but little variation in shape in large numbers of spores examined. (Cf. pl. 2, G.)

In structure the oogonium is characterized by the heavy uneven wall that led Schroeter to give the genus its distinctive name. On the outside this shows an irregular roughness, due partly to firmly adhering portions of the modified walls of disintegrated mesophyll cells among which the oogonia formed and partly to ridges developed on the wall of the growing oogonium as it was forced into the interstices between the surrounding cells. The true wall of the oogonium itself is thick but uneven, from 2 to even 10 μ in thickness, somewhat folded or irregularly dented and protruded, and of a hard, rather impermeable material greatly modified in composition from the original fungous cellulose. The inner surface of this wall is rather irregular in contour so that in some points it touches the spherical oospore inclosed and in others lies at a little space from it. (Pl. 2, G.) The wall of the oospore, however, is rather even, usually about 2 μ in thickness, smooth, refractive, and clear (pl. 2, G), and to various reagents reveals a different chemical composition from the wall of the oogonium surrounding it. Here and there the wall of the oogonium apparently has fused or become cemented to the wall of the inclosed oospore, whereas at other points there quite obviously is a gap between the outer surface of the oospore wall and the inner surface of the enveloping oogonium. After softening the oogonia with KOH and crushing them slightly, the oospores can be forced out entire in some instances, but ordinarily they remain securely inclosed by the oogonial wall even after lying in moist soil or soaking in water for weeks or months. Obviously, in the case of such bodies as these, in which the inclosing oogonium so closely envelops the single oospore within, the whole body must be considered as an entity and a resistant spore in its entirety quite as Schroeter pointed out in establishing the genus.

The size of these resting spores is a distinguishing feature which marks off this species clearly from most of the larger oogonial forms that have been described. In the size of the oogonium itself there is considerable variation, and because of the irregularity in shape of the whole structure, it is difficult and not particularly valuable in diagnosis to establish the size limit through quantitative measurements.

The inclosed oospore, however, shows much less variation in size, and since with proper illumination it can be seen even when the enveloping oogonial wall is thick and irregular, the diameters of the oospores can be measured with considerable accuracy. Such measurements of a large population of representative oospores furnish a good basis for comparing this species with others. The tabulation of frequency distribution of the various sizes encountered, as shown in Table 3, gives, therefore, a fair quantitative as well as qualitative conception of spore size as a diagnostic characteristic of the species.

TABLE 3.—*Measurements of diameters of the oospores of Sclerospora graminicola on Chaetochloa magna plants in Florida, tabulated both for separate collections made at different times in different localities and for the total*

Classes	Coco-plum Beach Road, 1924; number of oospores in 250	Key Biscayne, 1924; number of oospores in 250	Bird Road garden, 1924; number of oospores in 350	City of Sarasota, 1927; number of oospores in 150	Total number of oospores in 1,000
Diameter:					
19 to 20.9 μ	----------	1	----------	----------	1
21 to 22.9 μ	----------	1	1	----------	2
23 to 24.9 μ	----------	3	5	1	9
25 to 26.9 μ	3	6	7	6	22
27 to 28.9 μ	11	29	10	21	71
29 to 30.9 μ	36	66	37	31	170
31 to 32.9 μ	61	61	59	38	219
33 to 34.9 μ	46	45	58	29	178
35 to 36.9 μ	42	19	68	15	144
37 to 38.9 μ	27	15	55	6	103
39 to 40.9 μ	21	4	36	3	64
41 to 42.9 μ	1	----------	9	----------	10
43 to 44.9 μ	2	----------	5	----------	7

On comparing the measurements with those given by Schroeter (*13*), by Saccardo (*12*), by Fischer (*5*), by Berlese (*1*), by Traverso (*18*), by Butler (*2*), by Shirai (*15*), and by Ideta (*6*), they are found in general to agree, though it should be noted that most of the oospores measured by the writers fall within the size limits of 30 to 36 μ, which is not the average but rather the larger size limits of the relatively few measurements of the inclusive qualitative sort made by earlier investigators mentioned above.

In view of the fact that *Sclerospora graminicola* has such a diverse range of hosts and occurs so very widely distributed throughout the world, it would be of considerable interest to make an intensive comparative study of large numbers of oospores from many collections on different hosts and from different localities, to determine whether the species comprises strains with slight morphological differences in size and structure, as in the case of some other genera of downy mildews. Such a comparative investigation has been undertaken by the senior writer but has not progressed far enough to justify drawing conclusions as yet. It would be equally desirable to make a similar comparison of the zoosporangia, but as they should be measured at once when shed under optimum conditions, or at least should be carefully preserved under such conditions for future study, such an investigation is rather impracticable at present. The comparison in Table 2, however, between zoosporangia of this downy mildew on green foxtail grass in Minnesota in 1920 and on Everglade millet in Florida in 1924 shows no indication of morphologically distinct strains on these hosts in these two widely separate localities in the United States.

Although the structure, development, and general characteristics of the oogonia were determined without undue difficulty as they occurred on this new host in Florida, and although they have been described, at least in part, by other investigators of this species on

other hosts, the part these spores play in the life history of the fungus has not been worked out in all its details, and the actual method of germination has not been reported or investigated. As infection of seedlings in earth containing oospores was secured by the junior writer in the fall of 1924 in inoculation tests described later in this paper, it is justifiable to assume that germination of these bodies does take place in nature, and the evidence in the field is strong that it is occurring regularly in Florida under ordinary climatic conditions from year to year. The work of Melhus and Van Haltern (*9*) on the inoculation of various hosts by means of the resting spores, and more recent investigations by these authors and Bliss (*10*) on the hibernation and resistance of these bodies have added greatly to our knowledge of the oosporic phase of the fungus and have given information as to its activities which probably applies under Florida conditions as well as those of Ames, Iowa.

DISSEMINATION AND PERSISTENCE OF THE PARASITE

The dissemination of such fungi as this Sclerospora in general is of the two types recognized by Butler (*2*, *3*), a local noncontinuous spread by successive short flights and a long-distance spread in continuous uninterrupted journeys over great areas of land or water. In *Sclerospora graminicola* distribution of both types is known to occur, although in Florida it is chiefly the local spread from field to field and from locality to locality that seems to be taking place.

In the dissemination of the fungus the zoosporangia (conidia) and the zoospores emerging from them, the resting spores (consisting of the oospore within the enveloping oogonium), and even the mycelium itself may take part. In a study of the various aspects of the distribution and severity of the disease in Florida and the resultant measures for its control, the question of the structures taking part in dissemination and in persistence is of especial interest.

Local dissemination from plant to plant by the zoosporangia (conidia) apparently takes place very effectively. In Florida the production of abundant zoosporangia occurs practically throughout the year, for although it is at its maximum during the moist, cool nights of late winter and early spring, there is no time of year, at least in the southern part of the east coast, when in the infected clumps of grass one fails to find plants on which some zoosporangia are being produced in a practically continuous supply.

Dissemination of the fungus apparently is accomplished even more generally and successfully by means of the resting spores. These resting spores are produced, as has been said, in the parts of the plant in which the mycelium of the fungus is maturing, and as the grass clumps in any region are of different ages and new shoots are being sent out successively, there is a continuous production of the resting spores throughout the year, as there is of the zoosporangia, whose formation usually precedes them on the leaf surface.

Dissemination by means of mycelium within the host seemingly does not play any part in the case of this downy mildew, for the Everglade millet is propagated regularly not by stem cuttings which, as in the case of sugar cane, would contain living hyphae, but rather by seed. Apparently the mycelium does not become ensconced in

the seed, for when it grows so extensively in the head as to penetrate the developing ovaries these are badly deformed, and the seeds if formed at all are so imperfectly developed that they do not germinate.

The agents which accomplish dissemination of the fungus in the form of the zoosporangia and resting spores just considered are primarily wind and secondarily water. Although no special experiments were made to follow the distribution of the zoosporangia, this apparently involves the same general procedure as that already studied (*20*) in the case of the Philippine Sclerosporas, the conidia on maturing being wafted by the wind from the surface of the leaves on which they are produced and carried by moist night breezes to near-by plants. In the case of the resting spores wind is very active in distribution also. As the leaves in which the resting spores are maturing become shredded from the distintegration of the tissue between the fibrovascular bundles, these, being somewhat hygroscopic, twist and curl as they absorb moisture or as they dry out, effectively freeing the spores between them even on quiet days, and shaking with even the slightest jar of almost imperceptible breezes, so that a more or less abundant shower of resting spores is being scattered continually. Slides exposed under such plants caught an abundance of spores, and, as they are relatively small and light, it is justifiable to assume that they are carried away easily by the wind.

No matter how successfully these two types of reproductive bodies might be distributed, however, they would not achieve the spreading of the fungus unless they could accomplish the infection of healthy plants to which they might be blown. As has been noted, the zoosporangia are relatively short-lived and will not withstand drying or freezing or even brief exposure to strong sunlight. As they are produced at night, however, during abundant dew formation, their production occurs under conditions ideally suited for their being spread from plant to plant and reaching new hosts, still living and able to germinate and cause infection. These zoosporangia when caught on slides at night while they were being shed were found to germinate rapidly in pure water or in the dew that covered the leaves of near-by susceptible plants.

The resting spores, however, in spite of repeated observations, have not been seen to germinate, and their method of so doing and the exact process by which they accomplish infection are unknown. That they do germinate and do accomplish infection seems not to be doubted, however, both from observations in the field in Florida and from the following experiments by the junior writer. Series of tests were made in which healthy seeds were collected and planted in flats of sterilized soil with which had been mixed dried remains, 9 months old, of plants bearing an abundance of resting spores. The seeds germinated vigorously in about 8 days, and the seedlings appeared and reached a height of several inches in 2 weeks. When the seedlings were 4 weeks old they were examined carefully and 15 per cent were found to have been killed by the downy mildew, while 90 per cent of the survivors showed abundant infection. It is possible, of course, that resting mycelium in the dried plant parts mixed with the soil was responsible for this infection, but microscopic examination of such tissue gives no evidence that the mycelium is structurally

adapted to such resistance, and it seems much more probable that infection resulted from the germination of the resting spores thus introduced into the soil. It must be admitted, however, that careful searching and the examination of hundreds of resting spores taken from such soil or kept in hanging-drop cultures never revealed one that had germinated or showed any changes indicative of germination.

In addition to the means by which the fungus spreads from plant to plant and from place to place, the methods by which it persists from season to season and from year to year also are of great importance in their relation to the possible destructiveness of the disease in Florida. In connection with the persistence of the fungus from season to season, the same zoosporangia, resting spores, and mycelium concerned in its distribution must be considered.

The mycelium once ensconced in the tissue of the host remains there. In many cases, especially if the plant is fairly mature when attacked, the host is not killed but goes on developing and putting out new shoots, which in turn are invaded by the mycelium, so that this extension and this persistence of the parasite continue uninterrupted through the season. As has been noted, moreover, the Everglade millet, at least in the more southern parts of Florida, is not limited to a short growing season but is perennial, its clumps continuing to grow and to develop new suckers more or less vigorously from month to month and year to year. In such of these perennial clumps as are infected the persistent mycelium of the mildew also lives on indefinitely, running out into the newly developed shoots, where it sends forth successive crops of conidiophores on favorable nights and persisting and maturing resting spores throughout the tissue that is growing older. In some cases the infected plants seemed to be stimulated to much more active perennial growth than the uninfected, vigorously continuing to send out new shoots during the cold or dry periods when the activity of adjacent healthy plants was very slight. By the mycelium, therefore, the continuance of the mildew is very generally accomplished.

The zoosporangia are also instrumental in securing the persistence of the parasite from year to year under Florida conditions, for while they themselves are not resistant and do not survive long as such, they are produced in crop after crop by successive growth of the mycelium and continually accomplish infection and reinfection through the vicinity, thus serving as a means of perpetuating the disease.

Apparently, however, it is the resting spores especially that perpetuate the mildew from year to year. In contrast to the mycelium, which can not continue except as nourished by the host, and to the sporangia, which must reach and infect new hosts almost immediately, the resting spores are able to survive in plant remains and in the soil for considerable periods of time. The tissue-bearing resting spores which were mixed with earth for the infection experiments mentioned above had been dried in some cases for as long as 17 months and yet produced infection in the young seedlings in the same high percentage of cases as those more recently gathered. There is every evidence in the field that the part played by the resting spores is very important in the persistence of the disease in Florida. Obviously,

when one appreciates these various means by which the fungus is so efficiently distributed and perpetuated, it is easy to understand the persistently high percentage of infection of the Everglade millet throughout Florida. This matter also involves the question of the effectiveness of the methods by which infection is accomplished and of the rapidity with which infection is followed by spore formation and reinfection in building up an epidemic.

The precise method by which infection of the host plants is secured has not been studied in detail in Florida. It seems justifiable to assume, however, from what has been found to occur in the case of the Philippine Sclerosporas, that in this species also infection by means of the zoosporangia (conidia) and the zoospores emerging from them occurs, first, through the stomata in the young and tender tissue both of the curled-up terminal leaf and of the growing point within it surrounded by dew and exuded moisture, and, second, through the stomata of young leaf axils holding drops of dew. The manner in which the resting spores bring about infection has not yet been worked out, nor is it known whether the resting spores emit zoospores or send out a hypha that penetrates the host directly, or a limited mycelium which bears zoosporangia. The experiments by the junior writer reported here and the successful inoculation of maize and other hosts with these bodies by Melhus and Van Haltern (*9*) leave no doubt that infection does occur by means of resting spores. Moreover, it is clear from some of these cases that infection of germinating seedlings of the host plants had taken place even before they emerged from the ground.

The period of incubation elapsing between the time of infection and the production of zoosporangia or oogonia by the mycelium that has invaded the tissue is not known. In the experiments reported above, the seedlings showed infection and even produced sporangia in from 2 to 4 weeks after emerging from the soil containing resting spores. If one may judge from the case of the Philippine maize mildew, the incubation period following conidial infection would be even shorter, for infection of young seedlings by that mildew gave rise to mycelium which invaded the tissue very rapidly and in as short a time as 3 to 10 days produced abundant crops of spores on the surfaces of the young leaves. Even the longer period of 2 to 4 weeks, however, is sufficiently short to explain adequately the rapid increase in extent and amount of infection of the Everglade millet under Florida conditions.

PATHOGENICITY

The question whether the downy-mildew disease, so widespread, so virulent, and so successfully persistent on its wild host, the Everglade millet in Florida, may spread to valuable grasses cultivated as crops throughout the State is one of importance.

The crops which this species of downy mildew has been found to attack during the years it has been known in various parts of the world are chiefly Italian millet (*Chaetochloa* (*Setaria*) *italica* (L.) Scribn.) and the closely related Hungarian millet (*C. italica germanica* (Mill.) Scribn.), the bullrush or pearl millet (*Pennisetum typhoideum* L.), and the proso or broom millet (*Panicum miliaceum*

L.). The disease has been reported also as destructive to sorghum (*Andropogon sorghum* L.) in India, although it should be noted that there are strong reasons, which have been pointed out already (*20*), for believing that the fungus causing it is really a different species. Moreover, according to the not wholly convincing report of Massee (*8*), the disease has occurred, though not injuriously, on sugar cane (*Saccharum officinarum* L.) in England. Finally, the disease has been found to attack maize or Indian corn. This was first reported by R. B. Streets, who, in the summer of 1921, found the disease on sweet corn in Wisconsin and described his investigation of the situation in his report [5] to the Office of Cereal Crops and Diseases of the Bureau of Plant Industry. Recently Melhus and Van Haltern (*9*) have been successful in artificially infecting with *Sclerospora graminicola* not only the cultivated millets, *C.* (*Setaria*) *italica* and *P. miliaceum*, but also teosinte (*Euchlaena mexicana* Schrad.) and 26 varieties of maize.

In addition to these cultivated plants, the wild grasses, green foxtail (*Chaetochloa* (*Setaria*) *viridis*), and the smooth foxtail (*C. glauca*) have very generally been found to be attacked by this downy mildew wherever they occur in Europe and America.

Of all these host plants those that previous records show to be most susceptible, namely, the species of Chaetochloa, both the cultivated (the Italian millet and Hungarian millet) and the wild (the green foxtail and the smooth foxtail grass) are very rare in Florida and have not been available for examination.

In the case of pearl millet and sorghum, which are grown rather commonly in small patches, a careful examination of representative fields, chiefly around Miami, revealed no instances of attack by the downy mildew, even though plants of Everglade millet in all stages of infection by the fungus were growing abundantly in and around the cultivated areas.

Also, in the case of sugar cane, all the evidence gathered from field inspection indicates that the fungus certainly is not virulent to cane under field conditions in Florida. For example, along the edges of cane fields of plantations west of Miami, especially during the summer of 1924, there were great numbers of Everglade millet plants heavily infected with the downy mildew and obviously exposing to infection the young cane plants only a few feet away, yet careful search revealed no cases of downy mildew on the cane.

Furthermore, in the case of maize, a careful examination of fields in different districts in the State in which plants of the Everglade millet of all ages were growing in profusion as weeds, and were infected practically without exception with the actively sporulating downy mildew, did not reveal a single maize plant that was infected. In one cornfield near Miami, for example, the Everglade millet in 1925 was found growing abundantly as a weed among the corn, the grass even growing in the hills with the maize seedlings. When first examined the intermingled seedlings of the grass and the corn were young, some inches tall, in many instances with their leaves

[5] As this report and Streets's subsequent reports of his later survey work on this disease for the Office of Cereal Crops and Diseases were not published, they have escaped the attention they deserve, and the senior writer therefore has prepared a brief account of them, which is expected to appear shortly.

touching each other, and over 90 per cent of the grass seedlings were diseased with the downy mildew. Three weeks later 100 per cent of the grass seedlings were thus diseased, and in some cases the conidia of the fungus shed from the grass had fallen on the leaves of the corn in such numbers as to be obvious as a deposit of grayish color. The field was kept under observation until the corn was mature, yet during these three months not one corn plant was found infected with the mildew.

Not only did the disease fail to infect maize under field conditions, but, in the few attempts made, it failed also to transfer to maize when more directly exposed under artificial conditions. For example, in June, 1924, at Miami, seeds of yellow flint corn locally grown, when planted in tins in which heavily downy-mildewed plants of Everglade millet were grown for a study of the nocturnal production of conidia, developed into healthy seedlings, although from their first emergence they had been exposed repeatedly to showers of spores from the millet above them—a procedure that in the case of the Philippine Sclerosporas of maize would have resulted with certainty in a heavy percentage of infection.

The foregoing observations, of course, are too few and limited to be conclusive. They do indicate strongly, however, that cases of passage of the disease from Everglade millet to the crops mentioned are apparently very rare and certainly not numerous under Florida conditions.

ECONOMIC IMPORTANCE

At present the downy mildew as it exists in Florida is not of any great economic importance. The Everglade millet which is attacked is not sufficiently troublesome as a weed or sufficiently valuable as an uncultivated forage plant to make its destruction by the mildew either agriculturally a gain in the one case or a loss in the other.

However, the question to be considered is whether the disease in the future may not prove to be much more important. The present situation, that of a virulent disease with great potentialities for destruction, established on an abundant wild grass through all parts of the State, presents the alarming possibility that these infected areas of grass, although valueless in themselves, may serve as endemic foci of infection from which the disease may spread to related cultivated crops of great value, causing seriously destructive epidemics that could be controlled only at great cost. With this, as with other downy mildews, the destructiveness to various susceptible crops would depend not only on the power of the parasite to injure and destroy individual plants but also on the ability of the fungus to spread rapidly to large numbers of individuals. Obviously, in any given region such a disease might be rapidly fatal to individual plants yet not be of economic importance if local conditions did not permit the rapid spread to vast numbers of individuals; or a disease both severe and rapidly spreading would be relatively unimportant in a locality in which the crops thus attacked were of little value. In the case of this downy mildew in Florida, there is no doubt of its serious injury to individual plants, especially young ones, and no doubt of its effectiveness in rapid multiplication and spread. The question, therefore, is one of the present and possible future value

of the crops attacked. The Everglade millet on which it occurs is of no value in itself, as has been said, but its occurrence and that of the coextensive downy mildew upon it are so widespread throughout the State that it must always be considered as a potential reservoir of infection for susceptible cultivated crops.

As has been mentioned, the crops which have been reported as most susceptible to the disease elsewhere in the world are the Italian millet, the Hungarian millet, and the pearl millet, while there are records also of occasional occurrence on proso millet, sorghum, teosinte, sugar cane, and corn. Of these crops Italian millet and its variety, Hungarian millet, pearl millet, proso millet, sorghum, and teosinte are but little grown in Florida, and it does not seem probable that in the future they will be developed to such an extent that they will become of great value agriculturally.

Sugar cane, however, grown on the necessarily large acreages of a few recently developed sugar plantations, represents considerable capital, while corn, chiefly of the hard, small, yellow or white flint type, is very commonly grown throughout the State, the acreage and value in the aggregate making the crop of importance.

Practically everywhere that these susceptible crops are grown the Everglade millet grows also, either in patches of considerable extent in waste or abandoned land, along low bottoms or roadsides, or scattered in clumps and small groups as weeds in the cultivated fields themselves. And wherever the Everglade millet occurs the downy mildew is found on it, frequently attacking from 90 to 100 per cent of the plants and from season to season very efficiently persisting, reinfecting, and spreading.

Although many fields of these susceptible crops, with the exception of a few of the less common, have been examined carefully in various parts of the State, no cases of the spread of the disease to cultivated plants have been found.

It is conceivable that this downy mildew, which, like others, is very responsive to climatic conditions, might in the future during some extraordinarily favorable season transfer to related cultivated crops and become seriously destructive to them. This, however, seems very improbable. The disease shows every indication of having been established in Florida for a long time, yet there is no evidence of any such epidemic in the past. While the disease since its discovery in 1922 has been under special observation throughout the State under the wide range of seasonal conditions of the past five years, there have been found no cases of its even transferring to cultivated crops, much less threatening to become epidemic on them. If in the agriculture of the State the future brings some radical and unexpected change, such as the extensive development of the very susceptible German millet as a valuable large-scale crop, the disease may prove troublesome. Otherwise, considering all the evidence available, it seems to the writers logical to expect that the disease will continue to be as unimportant as it is at present.

CONTROL

From the fact that the Everglade millet on which the mildew occurs is of no especial value, from the fact that under field condi-

tions in Florida the fungus is rarely, if at all, pathogenic to commonly cultivated crops, and from the fact that the present status of the disease and the trend of agriculture in the State indicate that the disease is likely to continue to be of negligible economic importance, it follows that the problem of control is not an immediately pressing one. But because the fungus is so widely distributed throughout the State in centers of infection where it maintains itself on the Everglade millet successfully and in profusion year after year, it is safe to predict that its control, if ever it should become necessary, will be a matter of considerable difficulty.

SUMMARY

The downy mildew disease of the Everglade millet (*Chaetochloa magna*), caused by *Sclerospora graminicola*, was first encountered at Vero Beach, Fla., in November, 1922. Since then it has been found to be present on this grass, both growing wild in the Everglades and growing as a weed in cultivated land, in the following 14 counties: Dade, Broward, Palm Beach, Martin, St. Lucie, Indian River, Brevard, Volusia, Putnam, Duval, Alchua, Marion, Manatee, and Sarasota. This disease has never before been found in the southernmost States, nor has it ever been reported previously on this host.

Under Florida's subtropical conditions and on this grass the fungus runs through the same conidial and then oosporic stage characterizing it elsewhere on other hosts. The conidial phase shows the same development at night when the surface of the leaves is covered with dew or other moisture, follows the same schedule of emergence and development of the conidiophores, and agrees in the structure of the conidiophore and in the structural peculiarities, method of germination, and size characteristics of the conidia (zoosporangia). In like manner the oosporic phase agrees in the course of its development between the fibrovascular bundles until the leaves shred out to fibrous tangles, and in the essential structural features of the inclosing oogonia and the structure and size characteristics of the oospores within. Quantitative measurements of 250 conidia (zoosporangia) and of 1,000 oospores as given in tabular form.

The attack of the fungus usually results in the conspicuous systemic infection commonly reported, and from this the grass does not recover, new shoots being invaded successively as they sprout. Also there was found to occur a local type of infection, hitherto unreported, manifesting itself in an inconspicuous scattered spotting on the leaves. As this is not particularly noticeable, it seems probable that it may occur elsewhere on other hosts and have escaped attention previously. This local infection is of considerable importance in accomplishing dissemination of the disease. These two types of infection are illustrated and the pattern and extent of their sporulating areas are tabulated and compared.

The part played by the conidial and the oosporic phases of the disease in its local and long-distance spread, in its persistence from year to year, and in its injury to the host is considered, and the importance of the oospores is emphasized. Experiments are reported in which infection of Everglade millet seedlings was secured by

oospores even when these had rested previously more than nine months.

Although this mildew has been reported on other gramineous hosts, both wild and cultivated, throughout the world, careful searching in Florida for over four years has not revealed it on any host but the Everglade millet. Since, wherever this grass is known to occur in Florida, the fungus has been found on it, it is suggested that elsewhere throughout its range in the Gulf States and the West Indies the host will be found to harbor this mildew. Because this mildew hitherto has been observed in the United States only on wild or cultivated hosts introduced from Europe, it has been assumed that the fungus, originally described from Europe, is of the same origin, but the fact that it occurs so widely distributed in Florida on this wild-growing native grass is possible evidence against this assumption.

The disease, in nearly five years' observation, has not been found to be of any economic importance in Florida, nor has it given indication of probability that it will become so in the future, unless, perhaps, some change to valuable plantings of very susceptible crops takes place in the State. But, as the mildew has serious potentialities for destruction, it should be kept under observation.

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INHERITANCE OF RESISTANCE TO RUSTY BLOTCH IN BARLEY[1]

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INTRODUCTION

Rusty blotch of barley, although having much of the appearance of a physiological disease, has been definitely determined to be caused by a fungus identified as *Helminthosporium californicum* Mackie and Paxton (*10*).[3] Rusty blotch has spread from isolated areas to all parts of California in which barley is a major cereal crop. Damage from this disease is expressed in excessive leaf pruning, premature ripening, blasted florets, lodged plants, and shriveled grain. (Figs. 1 and 2.) The present paper deals with the inheritance of resistance to rusty blotch in a cross between a susceptible and an immune variety of barley. Breeding varieties resistant to disease is the only complete remedy now known for the control of rusty blotch of barley.

INHERITANCE OF RESISTANCE TO RUSTY BLOTCH IN A CROSS BETWEEN AN IMMUNE AND A SUSCEPTIBLE VARIETY OF BARLEY

After several years of experiment and observation, a variety of barley was found which for a number of years had shown no attack from rusty blotch. This barley, Chevalier, is a two-rowed variety included in the species *Hordeum distichon* (*11*). Chevalier was crossed with a very susceptible two-rowed variety, Abyssinian. Abyssinian is grouped by Harlan (*6*) under the species *H. deficiens* (*11*) and by Körnicke (*8*) under *H. distichon*, but by Beaven (*2*) under *H. decipiens* Steud.

Rusty blotch has occurred naturally at both Davis and Berkeley, where barley has been grown under close observation for years. In these localities Chevalier is apparently immune and Abyssinian is always susceptible.

The F_1 generation of the cross Abyssinian (susceptible) × Chevalier (immune) and the reciprocal were found to be completely free from rusty-blotch attack.

The F_2 generation was grown mainly at Berkeley because there late or spot blotch (*Helminthosporium sativum*) attacked with much less severity. Both rusty-blotch and spot-blotch lesions may, and frequently do, occur on the same leaves, rendering inspection for species disease attacks tedious and difficult. Blotches caused by mildew (*Erysiphe graminis* DC.) still further complicate a difficult situation. Mildew always attacks from the dorsal side. An examination of a leaf attacked

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[3] Reference is made by number (italic) to "Literature cited," p. 975.

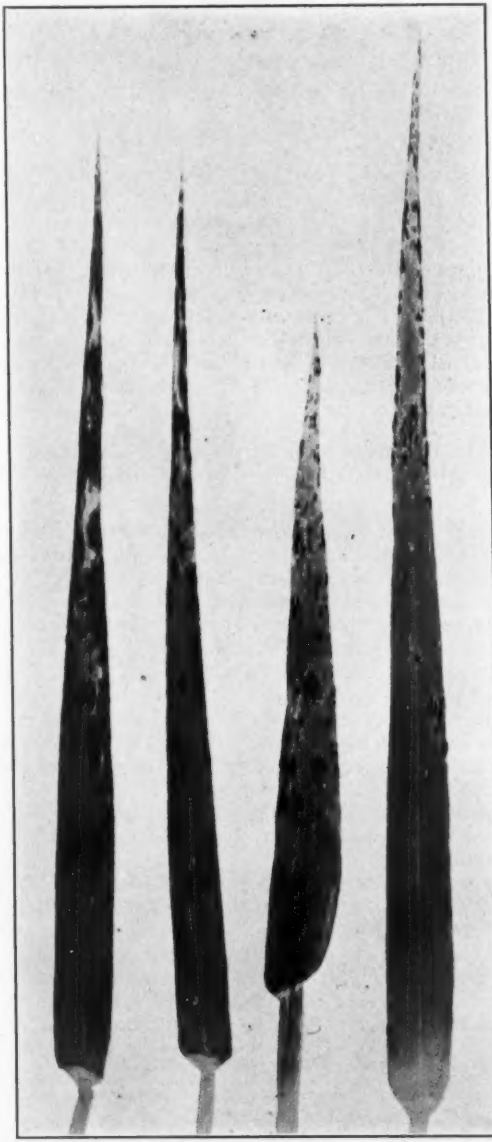

FIG. 1.—Rusty blotch on the dorsal and ventral sides of leaves on the Abyssinian variety of barley. Very late attack. Berkeley, Calif.

by mildew will show but slight browning on the ventral side beneath the mildew lesion. Rusty-blotch and late-blotch lesions are more equally intense in both dorsal and ventral sides of the leaf (*9*).

FIG. 2.—Rusty blotch on the upper leaves of barley, causing weakening of the culm and shriveling of the grain. El Toro, Orange County, Calif.

Because of the variability in number and size of rusty-blotch lesions no separation of susceptible hybrid plants into groups was made. The separation of F_2 plants into immune and susceptible groups is given in Table 1.

TABLE 1.—*Segregation of the 860 F_2 plants obtained in a cross between Chevalier, a variety immune to rusty blotch, and Abyssinian, a susceptible variety*

Item	Number of F_2 plants immune to rusty blotch	Number of F_2 plants attacked by rusty blotch
Observed	218.00	642
Expected	215.00	645
Deviation	+3.00	−3
Probable error	8.56	
D/P. E	.35	

In this table the inheritance of rusty-blotch resistance in the Mendelian ratio of three diseased to one disease-free plant is positively indicated, since the deviation is well within the limits of probable error. As immunity to rusty-blotch attack was completely dominant in F_1, the appearance of fixed recessive plants fully susceptible to rusty blotch is indicated. In the F_2 plants this segregation could only be indicated by intensity of attack, which is not positively reliable (*9*). Further tests of these F_2 plants were made, therefore, in the F_3 generation in plant-to-row tests. The results are presented in Table 2.

TABLE 2.—*Segregation of 275 F_3 families obtained in a cross between Chevalier, a variety immune to rusty blotch, and Abyssinian, a susceptible variety; plant-to-row tests in 8-foot rows*

Item	Number of F_3 families immune	Number of F_3 families susceptible	Number of F_3 families containing both immune and diseased plants
Observed	94.00	68.00	113.00
Expected (1:1:2)	68.75	68.75	137.50
Deviation	25.25	−.75	−24.50
Probable error	4.84	(?)	(?)
D/P. E	5.21	.15	5.00

As was expected, complete susceptibility appeared in the F_3 families as a pure recessive in an almost perfect 3:1 ratio, all plants in each of these rows being attacked by rusty blotch. In the group of immune families the deviation from the theoretical or expected ratio was great (+25). This wide deviation may be accounted for by the difficulties in detecting slight attacks of rusty blotch in the presence of heavy mildew infection and the occurrence of small lesions of spot blotch. The small number in the group of heterozygous families (−25) is accounted for by the misplacement of this number in the immune group.

INHERITANCE OF SPECIES CHARACTERS IN A CROSS BETWEEN HORDEUM DISTICHON AND H. DEFICIENS

The barley cross between Chevalier and Abyssinian made to study the inheritance of resistance to rusty blotch involved two species, according to Harlan's (*6*) classification. Chevalier, included in the

species *Hordeum distichon*, is a two-rowed barley with lateral florets possessing glumes of usual size reduced, blunt, but easily discernible lemma and palea, small but complete anthers, reduced ovary, and stigmas with infertile ovules. Abyssinian is also a two-rowed variety included in the species *Hordeum deficiens* in which the glumes alone of the lateral florets are normal in size. The lemma is much reduced and pointed, the palea is usually indiscernible, and only rudimentary traces of the sexual organs remain.

The F_1 plants in the cross Chevalier × Abyssinian and the reciprocals all gave the characteristic form of the *Hordeum distichon* parent except for the lemma, which was sharply pointed instead of blunt. This condition has been noted by Biffen (*3*), Engledow (*4*), and Gillis (*5*). The F_2 plants segregated for species inheritance into the groups presented in Table 3.

TABLE 3.—*Summary of the F_2 segregation of 860 plants obtained in cross of Hordeum distichon (Chevalier) × H. deficiens (Abyssinian)*

Item	Class 1, H. distichon type	Class 2, intermediate type	Class, 3 H. deficiens type
Observed	226.00	413.00	221.00
Expected ratio, 1:2:1	215.00	430.00	215.00
Deviation	+11.00	−17.00	+6.00
Probable error	8.56	8.56	8.56
D/P. E	1.28	1.98	.70

Little difficulty was encountered in placing the plants belonging to the parent groups, but the heterozygous plants showed a wide range of variation in the lateral florets. The subtending, or outer, glumes did not vary, but the lemma and palea varied not only in size but in shape. (Figs. 3 and 4.) Frequently heterozygous plants of the *Hordeum distichon* type produced greatly enlarged lemmas as compared with those normal for the *H. distichon* parent. Among the heterozygous plants resembling the *H. deficiens* parent many carried lemmas with blunt and rounded points instead of sharp pointed ones. The palea was frequently absent, but when present varied in size and shape. The sexual organs of these heterozygotes ranged from an entire absence of anthers and ovary to fully developed ones, but in no case were the lateral florets fertile. Fertility in all median florets was perfect in all phenotypic groups, no fertility being observed, as might possibly be expected, in species crosses.

The segregations fell into three classes, the two homozygous for the two parent species and the third or heterozygous group.

The Chevalier, or *Hordeum distichon* type, being recessive as shown in the F_1 generation, was readily separated and closely approximated the expected ratio. Greater difficulty was encountered in separating the homozygous plants of the Abyssinian (*H. deficiens*) type. This, in a measure, was due to the presence of small anthers in the heterozygous types (fig. 5) which were quite easily overlooked in the examination of so great a number of plants. It is evident, however, that a 1:2:1 Mendelian ratio showing a single-factor difference exists between the two species (*9*).

In order to verify the ratio obtained in the F_2 generation, seeds from these plants were sown during the following season in 8-foot rows. The segregation of the resulting families appears in Table 4.

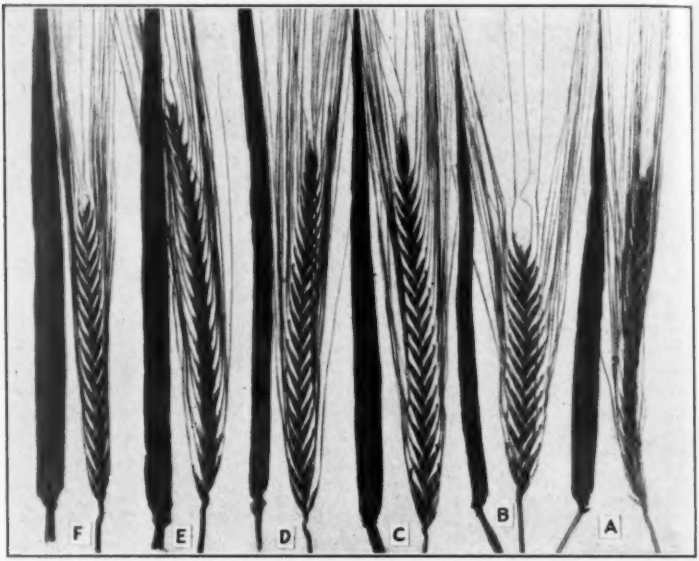

FIG. 3.—Parent plants and F_2 progeny of a cross between Chevalier, immune to rusty blotch, and Abyssinian, a susceptible variety: A, Chevalier (*H. distichon*) parent, immune to rusty blotch; B, Abyssinian (*H. deficiens*) parent, susceptible to rusty blotch; C, Abyssinian type (F_2), susceptible, spike long and lax like Chevalier; D, Abyssinian type (F_2), susceptible, long pointed lemmas; E, Chevalier type (F_2), susceptible, lateral florets much enlarged; F, Abyssinian type (F_2), no rusty blotch, lateral florets heterozygous type

TABLE 4.—*Segregation in 275 F_3 families according to species inheritance of a cross between Hordeum distichon and H. deficiens and the reciprocal*

Item	Number of F_3 families of H. distichon type	Number of F_3 families of H. deficiens type	Number of F_3 families of heterozygous type
Observed	65.00	77.00	133.00
Expected (1:2:1)	68.75	68.75	137.50
Deviation	−3.75	+8.25	−4.50
Probable error	4.84	4.84	4.84
D/P. E	.77	1.70	.93

The F_3 families segregated in the 1:2:1 ratio as shown in the F_2 plants. Those plants which were homozygous for the *Hordeum distichon* and the *H. deficiens* groups gave rise to homozygous families in F_3, while the heterozygous plants grouped into homozygous groups for each of the parent types and into heterozygous groups, as was to be expected. The same variability in size and shape of lemmas, presence and absence of palea, occurred as were observed in the F_2 plants.

CORRELATION BETWEEN RUSTY-BLOTCH ATTACK AND SPECIES INHERITANCE

In the F_1 plants of the cross Chevalier (*Hordeum distichon*, immune to rusty blotch) × Abyssinian (*H. deficiens*, susceptible) and reciprocals, immunity to rusty blotch and the *H. distichon* form appeared dominant. In the F_2 generation when the homozygous *H. deficiens*, the homozygous *H. distichon*, and the intermediate plants were each segregated in regard to their resistance or susceptibility to rusty blotch the distribution appears as shown in Table 5.

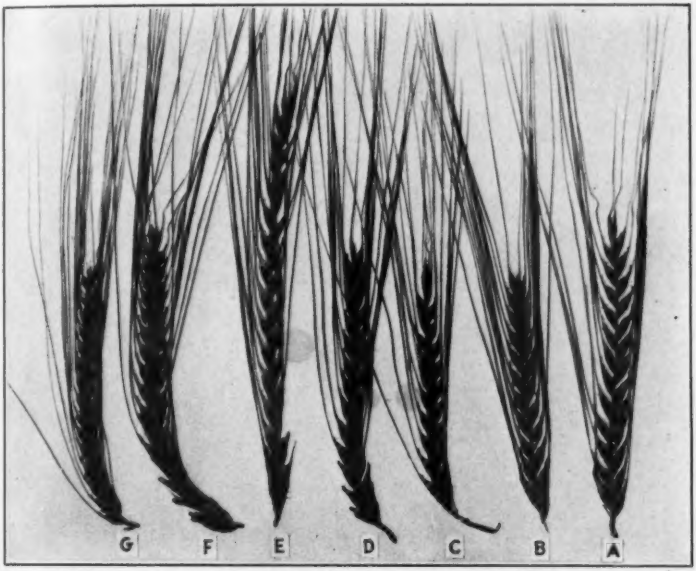

FIG. 4.—F_2 plants from a cross between Chevalier and Abyssinian: A, Spike lax like Chevalier but lemmas like Abyssinian, only larger and more pointed; B, spike dense and lateral florets like Abyssinian; C, spike dense like Abyssinian but lateral florets like Chevalier; D, spike dense like Abyssinian but lateral florets and lemmas like Chevalier; E, spike lax like Chevalier but florets intermediate in size and shape; F, spike with intermediate density but florets like Chevalier only much enlarged and infertile; G, spike dense like Abyssinian but florets like Chevalier, enlarged but infertile

TABLE 5.—*Rusty blotch attack in the F_2 plants of the homozygous and heterozygous groups*

Item	Class 1, H. distichon, homozygous			Class 2, intermediate or heterozygous			Class 3, H. deficiens, homozygous		
	Number of plants not attacked	Number of plants attacked	Total	Number of plants not attacked	Number of plants attacked	Total	Number of plants not attacked	Number of plants attacked	Total
Observed	173.000	53.000	226.00	309.00	86.00	395.00	146.000	75.000	221.00
Expected (1:2:1) (3:1)	157.875	52.625	210.50	315.75	105.25	421.00	157.875	52.625	210.50
Deviation	+15.125	+.375	+15.50	−6.75	−19.25	−26.00	−11.875	+22.375	+10.50
Probable error	4.390	4.390	8.47	5.80	5.80	8.47	4.340	4.340	8.47
D/P. E	3.400	.080	1.83	1.16	3.30	3.07	2.700	5.20	1.24

The results presented in Table 5 indicate that both the *Hordeum distichon-H. deficiens* contrast and resistance versus susceptibility to rusty blotch conform to the Mendelian law of segregation, inasmuch as the distribution of *H. distichon*, heterozygous, and *H. deficiens*

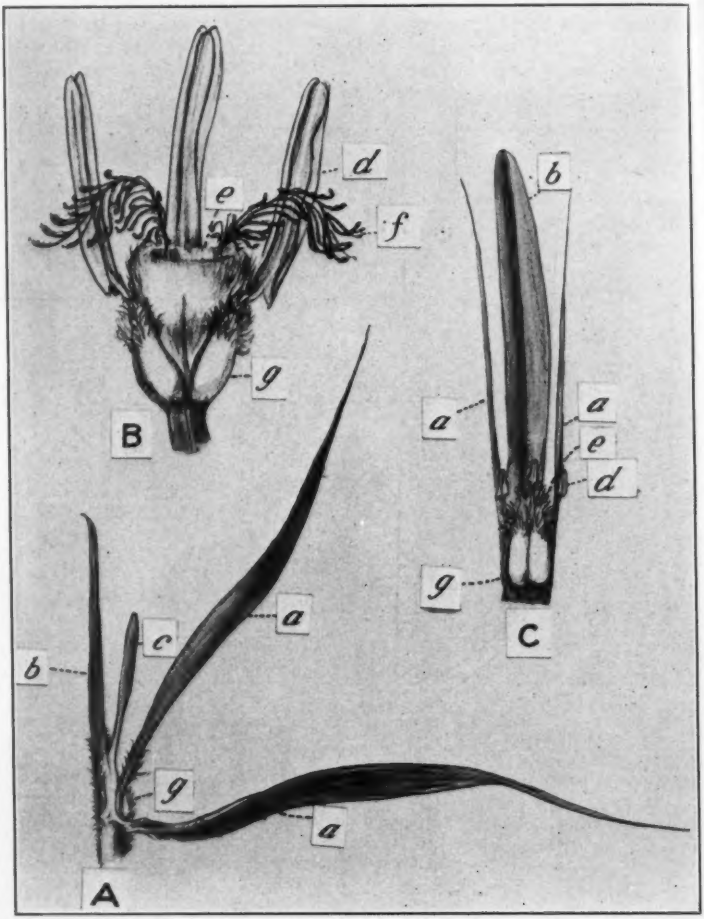

FIG. 5.—A, lateral floret of Abyssinian (*H. deficiens*); B, median floret of Chevalier (*H. distichon*); C, lateral floret of Chevalier: *a*, Glume; *b*, lemma; *c*, palea; *d*, anther; *e*, ovary; *f*, stigma; *g*, lodicule

plants closely approximates the 1:2;1 ratio, while in each of these 3 classes is found an approximation to the 3:1 ratio of resistant to susceptible plants. The theoretical distribution to be expected on the basis of segregation and independent assortment of two pairs of factors with incomplete dominance in one pair, using the conventional symbols *Aa* for *distichon* v. *deficiens*, and *Bb* for resistance v. susceptibility, would be $3AB$: $1Ab$: $6AaB$: $2Aab$: $3aB$: $1ab$. When

the observed distribution is compared with the theoretical numbers expected in case of such factorial relations, the deviations, as shown in Table 5, are rather wide, giving a value for D/E of more than 3 in 4 of the classes and of more than 5 in 1 class. Although considerable difficulty was experienced in classifying the *deficiens* plants which were attacked on account of the presence of other closely similar diseases, this does not explain the wide discrepancy in the case of *distichon* plants not attacked. It seemed worth while, therefore, to examine the observed distribution for the existence of genetic correlation. The data are assembled for comparison with the theoretical 9:3:3:1 distribution for two pairs of characters by combining the *H. distichon* and intermediate groups as is shown in Table 6.

TABLE 6.—*The data from Table 5 revised for comparison with the theoretical dihybrid distribution*

Item	Classes 1 and 2		Class 3	
	Not attacked	Attacked	Not attacked	Attacked
Observed	482.000	139.000	146.000	75.000
Expected (9:3:3:1	473.625	157.875	157.875	52.625
Deviation	+8.375	−18.875	−11.875	+22.375

The wide deviation from the theoretical 9:3:3:1 distribution shown in Table 6 at once suggests linkage between the two pairs of factors concerned. Applying the method of Babcock and Clausen (*1, p. 132–142*), the linkage value is found to be approximately 35. When, however, the observed distribution is compared with the theoretical distribution into six classes on the basis of 35 per cent of recombination in F_2, as shown in Table 7, the deviations are found to be just about as serious as those obtained when it was compared with the distribution expected on the basis of independent inheritance of the two pairs of factors. From the present data, therefore, it is impossible to decide whether or not there is genetic correlation between the *Hordeum distichon-H. deficiens* contrast and resistance v. susceptibility to rusty blotch. Until further data are available, however, it may be assumed that such correlation does not exist.

TABLE 7.—*Observed distribution in F_2 from the cross Hordeum distichon resistant ×H. deficiens susceptible, as compared with theoretical distributions in case of independent inheritance and linkage with 35 per cent of recombinations in F_2*

Phenotypic classes	Independent assortment	Observed distribution	35 per cent recombinations AB×ab	Phenotypic classes	Independent assortment	Observed distribution	35 per cent recombinations AB×ab
AB	157.875	173	184.7	Aab	105.250	86	95.7
Ab	52.625	53	25.8	aB	157.875	146	121.5
AaB	315.750	309	325.2	ab	52.625	75	88.9

A test of F_3 families grown in 1927 from F_2 plants was made. The observations are recorded in Table 8.

TABLE 8.—*Distribution in F_3 families of rusty-blotch resistance and susceptibility in homozygous and heterozygous groups*

Item	F_3 families of homozygous H. distichon group				F_3 families of heterozygous group				F_3 families of homozygous H. deficiens group			
	Number of rows total	Number of rows free of rusty blotch	Number of rows attacked by rusty blotch	Total	Number of rows total	Number of rows free of rusty blotch	Number of rows attacked by rusty blotch	Total	Number of rows total	Number of rows free of rusty blotch	Number of rows attacked by rusty blotch	Total
Observed	80.00	28.00	52.0	80.00	138.00	31.00	107.0	138.00	62.00	16.00	46.0	62.00
Expected	70.00	20.00	60.0	70.00	140.00	34.50	103.5	140.00	70.00	15.50	46.5	70.00
Deviation	+10.00	+8.00	−.8	+10.00	−2.00	−3.50	+3.5	−2.00	−8.00	+.50	−.5	−8.00
Probable error	4.89	2.61	------	4.89	4.89	3.43	------	5.64	4.89	2.30	------	4.89
D/P. E	2.04	3.06	------	2.04	.41	1.02	------	.35	1.63	.22	------	1.63

The expected 1:2:1 distribution for *Hordeum distichon* intermediate and *H. deficiens* is realized. The ratios of resistant to susceptible plants in these F_3 families are seriously disturbed by the very evident difficulty in detecting rusty-blotch attack when the lesions are small or obscured by mildew.

Undoubtedly some plants were genetically susceptible to rusty blotch but escaped attack. For these reasons rusty-blotch families observed are undoubtedly in excess of those genetically immune to attack. The homozygous *Hordeum deficiens* group has suffered a reduction, while the homozygous *H. distichon* group has been increased because of difficulties arising in the shape of the lemma, especially its point.

DISCUSSION

Species crosses usually result in partial or complete sterility in the offspring. Such behavior in hybrids may defeat the breeder working to create disease-resistant varieties. The two species involved in these breeding investigations gave, when crossed, complete fertility of the median florets. (Figs. 3 and 4.) The lateral florets are infertile, as both are two-rowed barleys (*Hordeum distichon* and *H. deficiens*). This finding is contrary to the findings of Biffen (*3*), Engledow (*4*), Hor (*7*), and Gillis (*5*), who encountered sterility to a greater or less degree in species crosses with barley, especially when six-rowed and two-rowed species were involved. Stimulation causing increase in length and width of lemma and palea in lateral florets when the two-rowed species *H. distichon* and *H. deficiens* were crossed has been recorded by Biffen (*3*), Engledow (*4*), Hor (*7*), and Gillis (*5*). Biffen found a single-factor difference to exist between these crosses, but the goodness of fit on which he based his 1:2:1 ratio was not close, 144 (28 per cent):259 (50.3 per cent):112 (21.7 per cent).

Agronomic characters for resistance to lodging and early maturity were studied, but no extensive counts were made. It appears from the evidence gathered that resistance to lodging (a character possessed by the Abyssinian parent) is dominant, as indicated by the F_1 and F_2 generations. Erect *v.* nodding heads—characters appearing in the Abyssinian and Chevalier parents, respectively—indicate that the erect head character is dominant in F_1 and F_2 generations. Early maturity was likewise found to be a dominant character.

SUMMARY

In crosses between Chevalier, an immune variety of barley, and Abyssinian, a susceptible variety, and their reciprocals, no rusty-blotch attack occurred in the F_1 plants.

In the F_2 generation the segregation occurred in the proportion of three nonattacked plants to one attacked, or in a 3:1 ratio, indicating a single factor difference for rusty-blotch resistance. F_3 families from the F_3 plants confirmed this ratio.

The two species involved in these crosses (*Hordeum distichon* and *H. deficiens*) gave in F_1 *H. deficiens* like plants, which gave rise in the F_2 generation to plants homozygous for both *H. distichon* and *H. deficiens* and intermediate in a Mendelian ratio of 1:2:1. This ratio was confirmed by the F_3 families from these F_2 plants.

No infertility was observed as a result of species crossing.

No decided evidence of linkage was found.

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